	L #	Hits	Search Text	DBs	Time Stamp
1	L1	1064	fructosyltransferase$1 or fructosyl adj transferase$1 or $sucrase	USPAT; US-PGPUB	2003/06/19 12:14
2	L2	17	1 same (lactobacillus or lactic adj acid adj bacteri$8)	USPAT; US-PGPUB	2003/06/19 12:14
3	L3	131	1 same (fructan or levan or inulin)	USPAT; US-PGPUB	2003/06/19 12:15
4	L4	73	1 same ((fructan or levan or inulin) near5 (mak$6 or produc$8 or synthes$8))	USPAT; US-PGPUB	2003/06/19 12:17
5	L5	86	2 or 4	USPAT; US-PGPUB	2003/06/19 12:18

PGPUB-DOCUMENT-NUMBER: 20030086959

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030086959 A1

TITLE: Animal feed containing simple polysaccharides

PUBLICATION-DATE: May 8, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Rhee, Sangki	Seoul		KR	
Song, Kibang	Taejeon		KR	
Kim, Chulho	Taejeon		KR	
Yoon, Byoungdae	Taejeon		KR	

APPL-NO: 10/ 169613

DATE FILED: July 3, 2002

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
KR	2000/000646	2000KR-2000/000646	January 7, 2000

PCT-DATA:
APPL-NO: PCT/KR00/01556
DATE-FILED: Dec 29, 2000
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 424/439

ABSTRACT:

The present invention relates to an animal feed containing the 0.04-2 (w/w) % simple polysaccharide on the basis of solid powder. The animal feed according to this invention is effective in improving the condition of evacuation, preventing the diarrhea and promoting the growth of animals.

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Summary of Invention Paragraph - BSTX (15):

[0012] As the practical aspect, levan in this invention is a polysaccharide consisting of .beta.-2,6 bonds between fructose molecules with 5-15% of .beta.-1,2 bond content in branch chains, and may be prepared from sugar using

levansucrase from microorganism such as Zymomonas mobilis, Bacillus sp., Rahnella aquatilis, and Gluconobacter sp., or plants Further, **levan may be produced** using recombinant **levansucrase,** whose coding gene has been purified, and transferred in microorganism such as E. coli, yeast or Bacillus subtilis, and which has been overexpressed

Summary of Invention Paragraph - BSTX (25):

[0021] 2000 units of **levansucrase** solution obtained in preparation 1 was added to 20 L of 25% sugar solution, and then the mixture had been incubated at 4.degree. C. for 7 days. **Levan concentration produced** in the reaction mixture was 43%, the saccharide composition was as following table 1

PGPUB-DOCUMENT-NUMBER: 20030049804

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030049804 A1

TITLE: Corynebacterium glutamicum genes encoding metabolic pathway proteins

PUBLICATION-DATE: March 13, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Pompejus, Markus	Freinsheim		DE	
Kroger, Burkhard	Limburgerhof		DE	
Schroder, Hartwig	Nussloch		DE	
Zelder, Oskar	Speyer		DE	
Haberhauer, Gregor	Limburgerhof		DE	
Kim, Jun-Won	Seoul		KR	
Lee, Heung-Shick	Seoul		KR	
Hwang, Byung-Joon	Seoul		KR	

APPL-NO: 09/ 746660

DATE FILED: December 22, 2000

RELATED-US-APPL-DATA:

child 09746660 A1 20001222

parent continuation-in-part-of 09606740 20000623 US PENDING

child 09746660 A1 20001222

parent continuation-in-part-of 09603124 20000623 US PENDING

non-provisional-of-provisional 60141031 19990625 US

non-provisional-of-provisional 60142101 19990702 US

non-provisional-of-provisional 60148613 19990812 US

non-provisional-of-provisional 60187970 20000309 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DE	19931420.9	1999DE-19931420.9	July 8, 1999

US-CL-CURRENT: 435/115, 435/183 , 435/252.3 , 435/320.1 , 435/69.1 , 536/23.2

ABSTRACT:

Isolated nucleic acid molecules, designated MP nucleic acid molecules, which encode novel MP proteins from Corynebacterium glutamicum are described. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing MP nucleic acid molecules, and host cells into which the expression vectors have been introduced. The invention still further provides isolated MP proteins, mutated MP proteins, fusion proteins, antigenic peptides and methods for the improvement of production of a desired compound from C. glutamicurn based on genetic engineering of MP genes in this organism.

RELATED APPLICATIONS

[0001] The present application is an continuation in part of U.S. patent application Ser. No. 09/606,740, filed Jun. 23, 2000. This application is also a continuation in part of U.S. patent application Ser. No. 09/603,124, filed Jun. 23, 2000. The present application claims priority to prior filed U.S. Provisional Patent Application Serial No. 60/141031, filed Jun. 25, 1999, U.S. Provisional Patent Application Serial No. 60/142101, filed Jul. 2, 1999, U.S. Provisional Patent Application Serial No. 60/148613, filed Aug. 12; 1999, U.S. Provisional Patent Application Serial No. 60/187970, filed Mar. 9, 2000, and also to German Patent Application No. 19931420.9, filed Jul. 8, 1999. The entire contents of all of the aforementioned applications are hereby expressly incorporated herein by this reference.

---------- KWIC ---------

Detail Description Table CWU - DETL (11):
7TABLE 4 ALIGNMENT RESULTS length % homology Date of ID # (NT) Genbank Hit Length Accession Name of Genbank Hit Source of Genbank Hit (GAP) Deposit rxa00657 906 GB_BA1:AF064700 3481 AF064700 Rhodococcus sp NO1-1 CprS and CprR genes, complete cds. Rhodococcus sp 40,265 15-Jul-98 metz 1314 GB_BA2:MTV016 53662 AL021841 Mycobacterium tuberculosis H37Rv complete genome, segment 143/162 Mycobacterium tuberculosis 61,278 23-Jun-99 metc 978 GB_BA2:CORCSLYS 2821 M89931 Corynebacterium glutamicum beta C-S lyase (aecD) and branched-chain amino acid Corynebacterium glutamicum 99,591 04-JUN-1998 upta rxa00023 3579 GB_EST33:AI776129 483 AI776129 EST257217 tomato resistant, Cornell Lycopersicon esculentum cDNA clone Lycopersicon esculentum 40,956 29-Jun-99 cLER17D3, mRNA sequence. GB_EST33:AI776129 483 AI776129 EST257217 tomato resistant, Cornell Lycopersicon esculentum cDNA clone Lycopersicon esculentum 40,956 29-Jun-99 cLER17D3, mRNA sequence. rxa00044 1059 EM_PAT:E11760 6911 E11760 Base sequence of **sucrase** gene. Corynebacterium glutamicum 42,979 08-OCT-1997 (Rel. 52, Created) GB_PAT:I26124 6911 I26124 Sequence 4 from U.S. Pat. 5556776. Unknown. 42,979 07-OCT-1996 GB_BA2:ECOUW89 176195 U00006 E. coli chromosomal region from 89.2 to 92.8 minutes. Escherichia coli 39,097 17-DEC-1993 rxa00064 1401 GB_PAT:E16763 2517 E16763 gDNA encoding aspartate transferase (AAT). Corynebacterium glutamicum 95,429 28-Jul-99 GB_HTG2:AC007892 134257 AC007892 Drosophila melanogaster chromosome 3 clone BACR02O03 (D797) RPCI-98 Drosophila melanogaster 31,111 2-Aug-99 02.O.3 map 99B-99B strain y; cn bw sp, *** SEQUENCING IN PROGRESS ***, 113 unordered pieces. GB_HTG2:AC007892 134257 AC007892 Drosophila melanogaster

chromosome 3 clone BACR02O03 (D797) RPCI-98 Drosophila melanogaster 31,111
2-Aug-99 02.O.3 map 99B-99B strain y; cn bw sp, *** SEQUENCING IN PROGRESS
***, 113 unordered pieces. rxa00072 rxa00105 798 GB_BA1:MTV002 56414
AL008967 Mycobacterium tuberculosis H37Rv complete genome; segment 122/162.
Mycobacterium tuberculosis 37,753 17-Jun-98 GB_BA1:ECU29581 71128 U29581
Escherichia coli K-12 genome; approximately 63 to 64 minutes. Escherichia coli
35,669 14-Jan-97 GB_BA2:AE000366 10405 AE000366 Escherichia coli K-12 MG1655
section 256 of 400 of the complete genome. Escherichia coli 35,669 12-Nov-98
rxa00106 579 GB_EST15:AA494237 367 AA494237 ng83f04.s1 NCI_CGAP_Pr6 Homo
sapiens cDNA clone IMAGE:941407 Homo sapiens 42,896 20-Aug-97 similar to
SW:DYR_LACCA P00381 DIHYDROFOLATE REDUCTASE;, mRNA sequence.
GB_BA2:AF161327
2021 AF161327 Corynebacterium diphtheriae histidine kinase ChrS (chrS) and
response Corynebacterium diphtheriae 40,210 9-Sep-99 regulator ChrA (chrA)
genes, complete cds. GB_PAT:AR041189 654 AR041189 Sequence 4 from U.S. Pat.
5811286. Unknown. 41,176 29-Sep-99 rxa00115 1170 GB_PR4:AC007110 148336
AC007110 Homo sapiens chromosome 17, clone hRPK.472_J_18, complete sequence.
Homo sapiens 36,783 30-MAR-1999 GB_HTG3:AC008537 170030 AC008537 Homo sapiens
chromosome 19 clone CIT-HSPC_490E21, *** SEQUENCING Homo sapiens 40,296
2-Sep-99 IN PROGRESS ***, 93 unordered pieces. GB_HTG3:AC008537 170030
AC008537 Homo sapiens chromosome 19 clone CIT-HSPC_490E21, *** SEQUENCING Homo
sapiens 40,296 2-Sep-99 IN PROGRESS ***, 93 unordered pieces. rxa00116 1284
GB_BA2:AF062345 16458 AF062345 Caulobacter crescentus Sst1 (sst1), S-layer
protein subunit (rsaA), ABC Caulobacter crescentus 36,235 19-OCT-1999
transporter (rsaD), membrane forming unit (rsaE), putative GDP-mannose-4,6-
dehydratase (IpsA), putative acetyltransferase (IpsB), putative perosamine
synthetase (IpsC), putative mannosyltransferase (IpsD), putative
mannosyltransferase (IpsE), outer membrane protein (rsaF), and putative
perosamine transferase (IpsE) genes, complete cds. GB_PAT:I18647 3300 I18647
Sequence 6 from U.S. Pat. 5500353. Unknown. 36,821 07-OCT-1996
GB_GSS13:AQ446197 751 AQ446197 nbxb0062D16r CUGI Rice BAC Library Oryza sativa
genomic clone Oryza sativa 38,124 8-Apr-99 nbxb0062D16r, genomic survey
sequence. rxa00131 732 GB_BA1:MTY20B11 36330 Z95121 Mycobacterium
tuberculosis H37Rv complete genome; segment 139/162. Mycobacterium
tuberculosis 43,571 17-Jun-98 GB_BA1:SAR7932 15176 AJ007932 Streptomyces
argillaceus mithramycin biosynthetic genes. Streptomyces argillaceus 41,116
15-Jun-99 GB_BA1:MTY20B11 36330 Z95121 Mycobacterium tuberculosis H37Rv
complete genome; segment 139/162. Mycobacterium tuberculosis 39,726 17-Jun-98
rxa00132 1557 GB_BA1:MTY20B11 36330 Z95121 Mycobacterium tuberculosis H37Rv
complete genome; segment 139/162. Mycobacterium tuberculosis 36,788 17-Jun-98
GB_IN2:TVU40872 1882 U40872 Trichomonas vaginalis S-adenosyl-L-homocysteine
hydrolase gene, complete Trichomonas vaginalis 61,914 31-OCT-1996 cds.
GB_HTG6:AC010706 169265 AC010706 Drosophila melanogaster chromosome X clone
BACR36D15 (D887) RPCI-98 Drosophila melanogaster 51,325 22-Nov-99 36.D.15 map
13C-13E strain y; cn bw sp, *** SEQUENCING IN PROGRESS ***, 74 unordered
pieces. rxa00145 1059 GB_BA1:MTCY2B12 20431 Z81011 Mycobacterium tuberculosis
H37Rv complete genome; segment 61/162. Mycobacterium tuberculosis 63,365
18-Jun-98 GB_BA1:PSEPYRBX 2273 L19649 Pseudomonas aeruginosa aspartate
transcarbamoylase (pyrB) and Pseudomonas aeruginosa 56,080 26-Jul-93
dihydroorotase-like (pyrX) genes, complete cds's. GB_BA1:LLPYRBDNA 1468
X84262 L. leichmannii pyrB gene. **Lactobacillus** leichmannii 47,514 29-Apr-97
rxa00146 1464 GB_BA1:MTCY2B12 20431 Z81011 Mycobacterium tuberculosis H37Rv
complete genome; segment 61/162. Mycobacterium tuberculosis 60,714 18-Jun-98

GB_BA1:MTCY154 13935 Z98209 Mycobacterium tuberculosis H37Rv complete genome; segment 121/162. Mycobacterium tuberculosis 39,229 17-Jun-98 GB_BA1:MSGY154 40221 AD000002 Mycobacterium tuberculosis sequence from clone y154. Mycobacterium tuberculosis 36,618 03-DEC-1996 rxa00147 1302 GB_BA1:MTCY2B12 20431 Z81011 Mycobacterium tuberculosis H37Rv complete genome; segment 61/162. Mycobacterium tuberculosis 61,527 18-Jun-98 GB_BA1:MSGB937CS 38914 L78820 Mycobacterium leprae cosmid B937 DNA sequence. Mycobacterium leprae 59,538 15-Jun-96 GB_BA1:PAU81259 7285 U81259 Pseudomonas aeruginosa dihydrodipicolinate reductase (dapB) gene, partial Pseudomonas aeruginosa 55,396 23-DEC-1996 cds, carbamoylphosphate synthetase small subunit (carA) and carbamoylphosphate synthetase large subunit (carB) genes, complete cds, and FtsJ homolog (ftsJ) gene, partial cds. rxa00156 1233 GB_BA1:SC9B10 33320 AL009204 Streptomyces coelicolor cosmid 9B10. Streptomyces coelicolor 52,666 10-Feb-99 GB_BA2:AF002133 15437 AF002133 Mycobacterium avium strain GIR10 transcriptional regulator (mav81) gene, Mycobacterium avium 54,191 26-MAR-1998 partial cds, aconitase (acn), invasin 1 (inv1), invasin 2 (inv2), transcriptional regulator (moxR), ketoacyl-reductase (fabG), enoyl-reductase (inhA) and ferrochelatase (mav272) genes, complete cds. GB_BA1:D85417 7984 D85417 Propionibacterium freudenreichii hemY, hemH, hemB, hemX, hemR and hemL Propionibacterium 46,667 6-Feb-99 genes, complete cds. freudenreichii rxa00166 783 GB_HTG3:AC008167 174223 AC008167 Homo sapiens clone NH0172O13, *** SEQUENCING IN PROGRESS ***, 7 Homo sapiens 37,451 21-Aug-99 unordered pieces. GB_HTG3:AC008167 174223 AC008167 Homo sapiens clone NH0172O13, *** SEQUENCING IN PROGRESS ***, 7 Homo sapiens 37,451 21-Aug-99 unordered pieces. GB_HTG4:AC010118 80605 AC010118 Drosophila melanogaster chromosome 3L/62B1 clone RPCI98-10D15, *** Drosophila melanogaster 38,627 16-OCT-1999 SEQUENCING IN PROGRESS ***, 51 unordered pieces. rxa00198 672 GB_BA1:AB024708 8734 AB024708 Corynebacterium glutamicum gltB and gltD genes for glutamine 2-oxoglutarate Corynebacterium glutamicum 92,113 13-Mar-1999 aminotransferase large and small subunits, complete cds. GB_BA1:AB024708 8734 AB024708 Corynebacterium glutamicum gltB and gltD genes for glutamine 2-oxoglutarate Corynebacterium glutamicum 93,702 13-MAR-1999 aminotransferase large and small subunits, complete cds. GB_EST24:AI232702 528 AI232702 EST229390 Normalized rat kidney, Bento Soares Rattus sp, cDNA clone Rattus sp 34,221 31-Jan-99 RKICF35 3' end, mRNA sequence. rxa00216 1113 GB_HTG2:HSDJ850E9 117353 AL121758 Homo sapiens chromosome 20 clone RP5-850E9, *** SEQUENCING IN Homo sapiens 37,965 03-DEC-1999 PROGRESS ***, in unordered pieces. GB_HTG2:HSDJ850E9 117353 AL121758 Homo sapiens chromosome 20 clone RP5-850E9, *** SEQUENCING IN Homo sapiens 37,965 03-DEC-1999 PROGRESS ***, in unordered pieces. GB_PR2:CNS01DSA 159400 AL121766 Human chromosome 14 DNA sequence *** IN PROGRESS *** BAC R-412H8 Homo sapiens 38,796 11-Nov-99 of RPCI-11 library from chromosome 14 of Homo sapiens (Human), complete sequence. rxa00219 1065 GB_HTG2:AC005079_0 110000 AC005079 Homo sapiens clone RG252P22, *** SEQUENCING IN PROGRESS ***, 3 Homo sapiens 38,227 22-Nov-98 unordered pieces. GB_HTG2:AC005079_1 110000 AC005079 Homo sapiens clone RG252P22, *** SEQUENCING IN PROGRESS ***, 3 Homo sapiens 38,227 22-Nov-98 unordered pieces. GB_HTG2:AC005079_1 110000 AC005079 Homo sapiens clone RG252P22, *** SEQUENCING IN PROGRESS ***, 3 Homo sapiens 38,227 22-Nov-98 unordered pieces. rxa00223 1212 GB_BA1:PPEA3NIF 19771 X99694 Plasmid pEA3 nitrogen fixation genes. Enterobacter agglomerans 48,826 2-Aug-96 GB_BA2:AF128444 2477 AF128444 Rhodobacter capsulatus molybdenum cofactor biosynthetic gene cluster, Rhodobacter capsulatus 40,135 22-MAR-1999 partial sequence. GB_HTG4:AC010111 138938 AC010111 Drosophila melanogaster chromosome 3L/70C1 clone RPCI98-9B18,

*** Drosophila melanogaster 39,527 16-OCT-1999 SEQUENCING IN PROGRESS ***, 64 unordered pieces. rxa00229 803 GB_BA2:AF124518 1758 AF124518 Corynebacterium glutamicum 3-dehydroquinase (aroD) and shikimate Corynebacterium glutamicum 98,237 18-MAY-1999 dehydrogenase (aroE) genes, complete cds. GB_PR3:AC004593 150221 AC004593 Homo sapiens PAC clone DJ0964C11 from 7p14-p15, complete sequence. Homo sapiens 36,616 18-Apr-98 GB_HTG2:AC006907 188972 AC006907 Caenorhabditis elegans clone Y76B12, *** SEQUENCING IN PROGRESS ***, Caenorhabditis elegans 37,095 26-Feb-99 25 unordered pieces. rxa00241 1626 GB_BA1:CGLYSI 4232 X60312 C. glutamicum lysI gene for L-lysine permease. Corynebacterium glutamicum 100,000 30-Jan-92 GB_HTG1:PFMAL13P1 192581 AL049180 Plasmodium falciparum chromosome 13 strain 3D7, *** SEQUENCING IN Plasmodium falciparum 34,947 11-Aug-99 PROGRESS ***, in unordered pieces. GB_HTG1:PFMAL13P1 192581 AL049180 Plasmodium falciparum chromosome 13 strain 3D7, *** SEQUENCING IN Plasmodium falciparum 34,947 11-Aug-99 PROGRESS ***, in unordered pieces. rxa00262 1197 GB_IN2:EHU89655 3219 U89655 Entamoeba histolytica unconventional myosin IB mRNA, complete cds. Entamoeba histolytica 36,496 23-MAY-1997 GB_IN2:EHU89655 3219 U89655 Entamoeba histolytica unconventional myosin IB mRNA, complete cds. Entamoeba histolytica 37,544 23-MAY-1997 rxa00266 531 GB_RO:AF016190 2939 AF016190 Mus musculus connexin-36 (Cx36) gene, complete cds. Mus musculus 41,856 9-Feb-99 EM_PAT:E09719 3505 E09719 DNA encoding precursor protein of alkaline

PGPUB-DOCUMENT-NUMBER: 20030044942

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030044942 A1

TITLE: Functional sugar polymers from inexpensive sugar sources and apparatus for preparing same

PUBLICATION-DATE: March 6, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Catani, Steven J.	Athens	GA	US	
Laurenzo, Kathleen S.	Athens	GA	US	
Navia, Juan L.	Athens	GA	US	
Walkup, Robert E.	Novi	MI	US	

APPL-NO: 10/ 127462

DATE FILED: April 23, 2002

RELATED-US-APPL-DATA:

child 10127462 A1 20020423

parent division-of 09305788 19990504 US GRANTED

parent-patent 6423833 US

non-provisional-of-provisional 60084281 19980505 US

US-CL-CURRENT: 435/91.2, 435/101 , 536/123 , 536/23.1

ABSTRACT:

A process for preparing functional sugar polymers comprising transferring a monosaccharide or oligosaccharide to an acceptor, removing by-products, separating polymers which have not achieved the desired chain length and recycling these underdeveloped polymers, and an apparatus for producing same.

---------- KWIC ---------

Detail Description Paragraph - DETX (25):

[0069] The system is also very flexible in how enzyme can be utilized. In one form, fresh enzyme is added as an ingredient and deactivated enzyme periodically removed using a membrane to recover any sugars carried with the purge. In other cases the organism which produces the enzyme can be grown in the reactor. This is the case when the organism can grow at the reaction

conditions and when it naturally over-expresses and excretes the required enzyme. In the case of **inulin, yeasts that produce** the fructose polymerization enzyme, e.g.; Aspergillus niger, A. japonicus Pullularia or Aureobasidium pullulans, Saccharomyces cerevisiae, etc., behave as just described. Further, they grow on the by-product of the reaction path, glucose. Processes which use **fructosyl-transferases** derived from Pullularia or Aureobasidium pullulans, and certain Aspergillus strains, useful in the preparation of fructans, are disclosed in the following: U.S. Pat. No. 4,309,505; U.S. Pat. No. 4,317,880; U.S. Pat. No. 4,335,207; U.S. Pat. Nos. 4,356,262; 4,423,150; U.S. Pat. No. 4,849,356.

Detail Description Paragraph - DETX (49):

[0081] A series of **inulin synthesis** reactions were run at 50 wt % and 28 units of **fructosyltransferase**/g carbohydrate with an 18 hour incubation at 50.degree. C. The carbohydrate composition of the starting material for the first reaction was 100% sucrose. The reaction was stopped by boiling and the final carbohydrate composition was analyzed by HPLC. The glucose byproduct was removed by chromatography. The remaining carbohydrates consisting of residual glucose, fructose, unreacted sucrose, and inulins of chain length DP3-7 were concentrated on a rotary evaporator and used as starting material in a s subsequent **inulin synthesis** reaction. The carbohydrate compositions of subsequent reactions were about 20% recycled carbohydrates and 80% sucrose. Eleven reactions total were run. Ten reactions contained recycled carbohydrates. The FIG. 6 shows the rise in polymer chain length with each reaction containing recycled carbohydrates.

PGPUB-DOCUMENT-NUMBER: 20030017600

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030017600 A1

TITLE: Double selection vector

PUBLICATION-DATE: January 23, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Gabant, Philippe	Brussels		BE	
Szpirer, Claude	Waterloo		BE	

APPL-NO: 10/ 168774

DATE FILED: June 20, 2002

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	998702682	1999EP-998702682	December 20, 1999

PCT-DATA:
APPL-NO: PCT/BE00/00151
DATE-FILED: Dec 20, 2000
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/472, 435/252.3 , 435/320.1

ABSTRACT:

The present invention is related to a nucleic acid construct (1) to be incorporated in a double selection vector (2) able to transform a cell (3) of a specific organism, wherein--said construct (1) contains two different genes (10 and 11), each gene encoding a different toxic molecule (4 and 5) to a prokaryote cell, preferably to E. Coli, said genes (10 and 11) being disposed upstream and downstream a cassette sequence (8), or downstream and upstream site(s) for the insertion of a cassette sequence (8), and--said nucleic acid construct comprises specific sequence portions (12, 12') allowing inactivation of said genes (10 and 11).

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (29):

[0025] Other examples of toxic molecules for a prokaryote cell are the protein encoded by the gene sacB (from Bacillus amylolique-faciens), the protein GpE, the protein GATA-1 or the protein Crp. The gene sacB encodes the **levan sucrase which catalyzes the hydrolysis of sucrose int pr ducts** which are toxic for E. Coli (Pierce et al. Proc. Natl. Acad. Sci., Vol. 89, N.degree.6 (1992) p. 2056-2060). The protein GpE encodes the E genes from the bacteriophage .phi.X174 which includes six unique restriction sites and encodes gpE and which causes lysis of E. Coli cell (Heinrich et al., Gene, Vol. 42 n.degree.3 (1986) p. 345-349). The protein GATA-1 has been described by Trudel et al. (Biotechniques 1996, Vol. 20(4), p. 684-693). the protein Crp has been described by Schlieper et al. (Anal. Biochem. 1998, Vol. 257(2), p. 203-209).

PGPUB-DOCUMENT-NUMBER: 20030003548

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030003548 A1

TITLE: Nucleotide sequences which code for the pck gene

PUBLICATION-DATE: January 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Eikmanns, Bernhard	Heidenheim		DE	
Riedel, Christian	New Ulm		DE	
Sahm, Hermann	Julich		DE	
Mockel, Bettina	Bielefeld		DE	

APPL-NO: 10/ 138713

DATE FILED: May 6, 2002

RELATED-US-APPL-DATA:

child 10138713 A1 20020506

parent continuation-in-part-of 10059091 20020130 US PENDING

child 10059091 20020130 US

parent division-of 09455777 19991207 US GRANTED

parent-patent 6420151 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DE	DE 199 50 409.1	1999DE-DE 199 50 409.1	October 20, 1999

US-CL-CURRENT: 435/106, 435/115 , 435/194 , 435/252.3 , 435/320.1 , 435/69.1 , 536/23.2

ABSTRACT:

The invention relates to isolated nucleotide sequences from Coryneform bacteria which code for the pck gene encoding the enzyme phosphoenol pyruvate carboxykinase (PEP carboxykinase). The invention also relates a process for the fermentative preparation of L-amino acids, in particular L-lysine, L-threonine, and L-glutamate by attenuation of the pck gene.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a Continuation-in-Part of U.S. application Ser.

No. 10/059,091, filed Jan. 30, 2002, which, in turn, is a Divisional of U.S. application Ser. No. 09/455,777, filed Dec. 7, 1999. U.S. application Ser. No. 09/455,777 claims priority to German application 199 50 409.1 filed on Oct. 20, 1999.

---------- KWIC ---------

Detail Description Paragraph - DETX (86):

[0115] E. coli S17-1 was then transformed with the integration plasmid pK19mobsacB.DELTA.pck (Simon et al., Bio/Technology 1,784-791 (1983)). This strain allows transfer of a plasmid to Corynebacterium glutamicum by conjugation (Schfer et al., Journal of Bacteriology 172 (1990) 1663-1666). The lysine production strain C. glutamicum MH20-22B was used as the recipient of the conjugation (Schrumpf et al., Applied Microbiology and Biotechnology 37 (1992) 566-571)). Several transconjugants were obtained from the conjugation between E. coli S17-1/pk19mobsacB.DELTA.pck and C. glutamicum MH20-22B and subsequent selection on Luria-Bertani agar plates with kanamycin (25 .mu.g/ml) and nalidixic acid (50 .mu.g/ml). For selection for the second recombination event, which is to lead to excision of the vector together with the pck gene, these transconjugants were cultured on antibiotic-free Luria-Bertani complex medium [Sambrook et al; Molecular Cloning, A laboratory manual (1989) Cold Spring Harbor Laboratory Press] with 1% glucose and then plated out on the same medium plus 10% sucrose. The sacB gene present on the vector pk19mobsacB codes for the enzyme **levan sucrase and leads to synthesis of levan** from sucrose. Since levan is toxic to C. glutamicum, only C. glutamicum cells which have lost the integration plasmid can grow on sucrose-containing medium (Jger et al., Journal of Bacteriology 174 (1992) 5462-5466). 30 sucrose-resistant clones were investigated for their kanamycin sensitivity. For 11 of the clones tested, in addition to the sucrose resistance, the desired kanamycin sensitivity could also be confirmed. In these 11 clones, the vector background had therefore been excised again. Whether the desired deletion had also taken place was tested by analysis by means of the polymerase chain reaction (PCR). For this, chromosomal DNA was liberated from a colony of the starting strain and from colonies of the 11 kanamycin-sensitive clones. For this, the particular colony was removed from the agar plate with a toothpick, suspended in 50 .mu.l H.sub.2O and incubated for 5 minutes at 95.degree. C. 1 .mu.l portions of the resulting solution were each employed as templates in the PCR. Oligonucleotides which cover the regions from nucleotide 2136 to 2158 and from 3815 to 3793 in SEQ ID No. 1 were used as primers. The PCR conditions were: prior denaturing: 150 seconds at 94.degree. C.; denaturing 60 seconds at 94.degree. C.; hybridization 30 seconds at 60.degree. C.; amplification 120 seconds at 72.degree. C.; 30 cycles, end extension 240 seconds at 72.degree. C. On the basis of the primers chosen, a PCR product of 1.68 kb was expected in the batch with the DNA of the starting strain. A PCR product of 0.61 kb was expected in the PCR with the pck deletion mutant. A PCR product 0.61 kb in size was obtained with one clone. The desired deletion of the internal 1071 bp pck fragment in this clone was thereby demonstrated. The clone was called MH20-22B.DELTA.pck. The 1.68 kb PCR product was detected in the batches of the other clones. In these, the vector had thus been excised such that the genomic starting situation was re-established.

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DOCUMENT-IDENTIFIER: US 20020170092 A1

TITLE: Modification of polysaccharides

PUBLICATION-DATE: November 14, 2002

INVENTOR-INFORMATION:

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APPL-NO: 10/ 112797

DATE FILED: March 29, 2002

RELATED-US-APPL-DATA:

child 10112797 A1 20020329

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child 09117232 19990301 US

parent a-371-of-international PCT/NL97/00039 19970207 WO UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
NL	1002275	1996NL-1002275	February 7, 1996

US-CL-CURRENT: 800/284, 435/101 , 536/123

ABSTRACT:

The present invention relates to a method for manufacturing modified polysaccharide in contact with a sugar group transferring enzyme and a sugar group donor. The result of the method is modified polysaccharides, which can be used for different food and non-food applications.

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Detail Description Paragraph - DETX (11):

[0057] Determination of the activity of the starch or the cellulose by scintillation count revealed that the activity of the material incubated in the presence of **fructosyl transferase** was respectively 8 and 19 times higher than the activity of the material added after the 18 hour incubation period at 30.degree. C. of the levan **sucrase** enzyme (table.2), while TLC analysis

revealed that the total **fructan synthesis** was constant in all reaction mixtures. This demonstrates that the levan **sucrase** enzyme of Bacillus subtilis is indeed capable of using starch and cellulose as fructosyl acceptor in the transfructosylation reaction.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020170086 A1

TITLE: Fructan biosynthetic enzymes

PUBLICATION-DATE: November 14, 2002

INVENTOR-INFORMATION:

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APPL-NO: 10/ 003392

DATE FILED: October 30, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60244273 20001030 US

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US-CL-CURRENT: 800/278, 435/320.1 , 435/419 , 536/23.6

ABSTRACT:

This invention relates to isolated nucleic acid fragments encoding fructosyltransferases. More specifically, this invention relates to polynucleotides encoding 1-FFTs, 6-SFTs, or 1-SSTs. The invention also relates to the construction of a recombinant DNA constructs encoding all or a portion of the fructosyltransferases, in sense or antisense orientation, wherein expression of the recombinant DNA construct results in production of altered levels of the fructosyltransferases in a transformed host cell.

[0001] This application claims the benefit of U.S. Provisional Application No. 60/244,273, filed Oct. 30, 2000, and U.S. Provisional Application No. 60/269,543, filed Feb. 16, 2001. The entire contents of these two applications are herein incorporated by reference.

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Summary of Invention Paragraph - BSTX (5):

[0004] Based on their structure, several types of fructans can be identified in higher plants. The most characterized plant fructan is inulin. Inulin contains linear .beta.(2-1)-linked fructosyl residues and commonly occurs in

the Asterales such as Jerusalem artichoke (Helianthus tuberosus), sunflower (Helianthus sp.), Belgian endive (Cichorium intybus) and artichoke (Cynara scolymus). **Inulin synthesis** is initiated by sucrose:sucrose 1-**fructosyltransferase** (1-SST; EC 2.4.1.99) which catalyses the conversion of sucrose into isokestose (also named 1-kestose) and glucose. Additional fructosyl units are added onto isokestose, by the action of a fructan:fructan 1-**fructosyltransferase** (1-FFT, EC 2.4.1.100) resulting in a .beta.(2-1)-linked fructose oligomer.

Summary of Invention Paragraph - BSTX (6):

[0005] A second type of fructan is called levan and consists of linear .beta.(2-6) linked fructosyl residues. Grasses such as Dactylis glomerata and Phleum pratense contain levans with a DP up to 200. Levans are **synthesized by a sucrose:fructan** 6-**fructosyltransferase** (6-SFT; EC 2.4.1.10) that uses sucrose as a fructosyl donor and acceptor to produce 6-kestose. Polymerization of 6-kestose is believed to be catalyzed by 6-SFT as well, using sucrose as the fructosyl donor.

Summary of Invention Paragraph - BSTX (8):

[0007] The fourth type of fructan is often referred to as the neo-kestose series of fructans. The neo-kestose series have fructosyl residues on the carbon 1 and 6 of glucose producing a polymer with fructosyl residues on either end of the sucrose molecule. The inulin-neoseries found in Liliales such as onion (Allium cepa), leek (Allium porrum), and asparagus (Asparagus officinales) contain mainly a .beta.(2-1)-linked fructose polymer linked to carbon 1 and 6 of glucose, while the levan-neoseries contain mainly a .beta.(2-6)-linked fructose polymer linked to carbon 1 and 6 of glucose. Neoseries fructans are believed to be synthesized by the concerted action of 1-SST (**producing isokestose) and 6G-FFT, a specific fructan**:fructan 6G-**fructosyltransferase** that polymerizes fructosyl units onto carbon 6 of glucose.

Summary of Invention Paragraph - BSTX (29):

[0027] In an eighteenth embodiment, this invention relates to a method of altering the level of expression of a fructan biosynthetic enzyme (**fructosyltransferase**) polypeptide in a host cell comprising: (a) transforming a host cell with a recombinant DNA construct of the present invention; and (b) growing the transformed host cell under conditions that are suitable for expression of the recombinant DNA construct wherein expression of the recombinant DNA construct results in **production of altered levels of the fructan** biosynthetic enzyme (**fructosyltransferase**) polypeptide in the transformed host cell.

Detail Description Paragraph - DETX (36):

[0066] As was noted above, the nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed polypeptides are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have

the effect of altering the fructan profile in those cells. Nucleic acid fragments encoding the fructan biosynthetic enzymes (**fructosyltransferases**) disclosed herein may be used to generate trangenic plants that produce particular fructans. In particular, the ability to produce fructans of the desired size in large amounts in crops of agronomic importance, such as corn or soybean, will reduce **fructan production** costs.

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DOCUMENT-IDENTIFIER: US 20020155568 A1

TITLE: Novel glucosyltransferases

PUBLICATION-DATE: October 24, 2002

INVENTOR-INFORMATION:

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DATE FILED: November 29, 2001

RELATED-US-APPL-DATA:

child 09995749 A1 20011129

parent continuation-in-part-of 09604957 20000628 US PENDING

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	00201871.1	2000EP-00201871.1	May 25, 2000

US-CL-CURRENT: 435/193, 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

The present invention describes a protein having glucosyltransferase activity. This protein is derived from lactobacilli, which are food-grade microorganisms with the Generally Recognized As Safe (GRAS) status. The protein produces a glucan with a unique structure having 4-linked, 6-linked and 4,6-linked anhydroglucose units or in the presence of suitable acceptors, oligosaccharides. According to the invention lactobacilli capable of producing this glucan using the novel glucosyltransferase can be used as a probiotic or symbiotic.

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a continuation-in-part application of U.S. application Ser. No. 09/604,957 filed on Jun. 28, 2000, which claims priority from European Application No. 00201871.1 filed on May 25, 2000.

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Summary of Invention Paragraph - BSTX (10):
[0008] It was found according to the invention that the glucans are produced by certain **Lactobacillus** strains, in particular by certain strains of **Lactobacillus** reuteri, as a result of the activity of a single glucosyltransferase (**glucansucrase**).

Detail Description Paragraph - DETX (6):
[0031] The glucosyltransferase (gtfA) gene was identified by amplification with PCR using degenerated primers (GTFprl, 5'GAYAAKWSNAAKSYNRTNGTNSARGC3- ' (SEQ ID No. 6) and GTFpr2, 5'GNKCNCANATRATRCCNCTRNA3' (SEQ ID No. 7); Y=T or C, K=G or T, W=A or T, S=C or G, R=A or G, N=A, C, G, or T) based on conserved amino acid sequences deduced from different glucosyltranferase genes (gtfS of Streptococcus downei, gtfC of S. mutans, gtfl of S. downei, gtfK and gtfM of S. salivarius and dsrA of Leuconostoc mesenteroides) and **Lactobacillus** reuteri chromosomal DNA as template. An amplification product with the predicted size of about 660 bp was obtained (FIG. 1A). To investigate the possible presence of multiple copies of the glucosyltransferase gene, Southern hybridization was performed. DNA was restricted with endonucleases, separated by agarose gel electrophoresis and transferred to a Hybond nylon membrane. For hybridization, probes were labelled wih [.alpha.-.sup.32P]dCTP using Random Primed DNA labeling kit (Boehringer Mannheim), following the manufacturer's instructions. The Southern hybridization of chromosomal DNA of the **Lactobacillus** reuteri strain 121 with the amplified 660 bp PCR fragment, followed by washing under non-stringent conditions (45.degree. C., 0.5x SSC/0.1 SDS) revealed one hybridizing fragment, suggesting the presence of only a single copy of a glucosyltransferase gene in the **Lactobacillus** reuteri strains. The 660 bp fragment was cloned in E. coli JM1O9 using the pCR2.1 vector. Transformations were performed by electroporation using the BioRad gene pulser apparatus at 2.5 kV, 25 .mu.F and 200 .OMEGA., following the instructions of the manufacturer. The fragment was sequenced by the method of Sanger et al. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467, confirming that the correct part of the gtfA gene had been isolated. The 660 bp amplified fragment was used to design primers for inverse PCR. Using inverse PCR techniques a 3 kb fragment of the isolated gtfA gene was generated (FIG. 1B). This 3 kb amplicon was identified by sequencing and probes were designed to isolate the EcoRI/BglII and EcoRI/HindIII fragments from a partial DNA library of **Lactobacillus** reuteri in E. coli DH5.alpha. (FIG. 1C). Positive clones were selected by colony blot hybridization using Hybond-N filters, following the instructions of the supplier and the cloned fragments were sequenced. Attempts to clone the C-terminal part of the **glucansucrase** gene in E. coli DH5.alpha. or JM109 using a partial DNA library strategy with different vectors failed. Therefore, the C-terminal part was isolated by inverse PCR. The remaining fragment, located between the EcoRI/BglII and EcoRI/HindIII fragments, was isolated by PCR techniques (FIG. 1D). The amplicons obtained were sequenced directly. To eliminate errors due to the PCR reaction, these fragments were sequenced for at least 4 times, using different clones per PCR reaction. Both DNA strands of the entire glucosyltransferase gene were sequenced twice. In this way the

sequence of a 5.5 kb region of the **Lact bacillus** reuteri chromosomal DNA, containing the gtfA gene and its surroundings, were obtained.

Detail Description Paragraph - DETX (24):

[0046] FIG. 4: Alignment of catalytic cores of **alternansucrase** (ASR) of Leuconostoc mesenteroides strain NRRL B-1355 **dextransucrase** (DSRS) of Leuconostoc mesenteroides strain NRRL B-512F, glucosyltransferase-D (GTFD) of Streptococcus mutans GS5, glucosyltransferase-A of **Lactobacillus** reuteri and **amylosucrase** (AS) of Neisseria polysaccharea. * indicates identical or conserved residues in all sequences); - - - , gap in the sequence; AA amino acids which are conserved in all other glucosyltranferases but not in GTFA; , putative catalytic residues; .circle-solid., putative calcium binding sites; .diamond-solid., putative residues stabilizing the transition state; .gradient., residues possibly playing a role in binding of acceptor molecules and in the transfer of the glucosyl residue; .diamond., putative chloride binding sites; -Ex-, localization of .beta.-strands; -Hx-, localization of .alpha.-helices according to Mac Gregor et al. (1996) FEBS Lett. 378, 262-266. The numbering of the amino acids of the glucosyltransferase-A of **Lactobacillus** reuteri corresponds to the positions of these amino acids in the amino acid sequence 531-1781 of amino acid sequence SEQ ID No. 2, when the amino acid sequence 531-1781 is renumbered 1-1251.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020155557 A1

TITLE: Nucleotide sequences which code for the rpsL gene

PUBLICATION-DATE: October 24, 2002

INVENTOR-INFORMATION:

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DATE FILED: February 15, 2002

FOREIGN-APPL-PRIORITY-DATA:

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DE	101 07 230.9	2001DE-101 07 230.9	February 16, 2001
DE	101 62 386.0	2001DE-101 62 386.0	December 19, 2001

US-CL-CURRENT: 435/115, 435/199 , 435/252.3 , 435/320.1 , 435/69.1 , 536/23.2

ABSTRACT:

An isolated polynucleotide comprising a polynucleotide sequence chosen from the group consisting of
a) polynucleotide which is identical to the extent of at least 70% to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID No. 2,
b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least 70% to the amino acid sequence of SEQ ID No. 2,
c) polynucleotide which is complementary to the polynucleotides of a) or b), and
d) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequence of a), b) or c),
and a process for the fermentative preparation of L-amino acids using coryneform bacteria in which at least the rpsL gene is present in enhanced form, as well as the use of polynucleotides which comprise the sequences according to the invention as hybridization probes.

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Detail Description Paragraph - DETX (128):

[0163] The plasmid pK18mobsacB_rpsL-1545, like the starting plasmid pK18mobsacB, contains, in addition to the kanamycin resistance gene, a copy of the sacB gene which codes for levan **sucrase** from Bacillus subtilis. The expression which can be induced by sucrose leads to the formation of **levan sucrase, which catalyses the synthesis of the product levan,** which is toxic to C. glutamicum. Only those clones in which the integrated pK18mobsacB_rpsL-1545 has excised as the consequence of a second recombination event therefore grow on LB agar. Depending on the position of the second recombination event with respect to the mutation site, allele exchange or incorporation of the mutation takes place with the excision, or the original copy remains in the chromosome of the host.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020127681 A1

TITLE: Novel fructosyltransferases

PUBLICATION-DATE: September 12, 2002

INVENTOR-INFORMATION:

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APPL-NO: 09/ 995587

DATE FILED: November 29, 2001

RELATED-US-APPL-DATA:

child 09995587 A1 20011129

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COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	00201872.9	2000EP-00201872.9	May 25, 2000

US-CL-CURRENT: 435/193, 435/101 , 435/252.3 , 435/325 , 435/69.1 , 536/123 , 536/23.2

ABSTRACT:

The present invention describes two novel proteins having **fructosyltransferase** activity. Both enzymes are derived from lactobacilli, which are food-grade micro-organisms with the Generally Recognized As Safe (GRAS) status. One of these proteins **produces an inulin and fructo-oligosaccharides, while the other produces a levan** and fructo-oligosaccharides. According to the invention lactobacilli capable of **producing an inulin** and/or a levan and/or fructo-oligosaccharides using one or both of the **fructosyltransferases** can be used as a probiotic or a symbiotic.

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a continuation-in-part application of U.S. application Ser. No. 09/604,958 filed on Jun. 28, 2000, which claims priority

from European Application No. 00201872.9 filed on May 25, 2000.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):

The present invention describes two novel proteins having **fructosyltransferase** activity. Both enzymes are derived from lactobacilli, which are food-grade micro-organisms with the Generally Recognized As Safe (GRAS) status. One of these proteins **produces an inulin and fructo-oligosaccharides, while the other produces a levan** and fructo-oligosaccharides. According to the invention lactobacilli capable of **producing an inulin** and/or a levan and/or fructo-oligosaccharides using one or both of the **fructosyltransferases** can be used as a probiotic or a symbiotic.

Summary of Invention Paragraph - BSTX (6):

[0006] The exopolysaccharides produced by LAB can be divided in two groups, heteropolysaccharides and homopolysaccharides; these are synthesized by totally different mechanisms. The former consist of repeating units in which residues of different types of sugars are present and the latter consist of one type of monosaccharide. The synthesis of heteropolysaccharides by **lactic acid bacteria,** including lactobacilli, has been studied extensively in recent years. Considerably less information is available on the synthesis of homopolysaccharides from lactobacilli, although some studies have been performed. Homopolysaccharides with fructose as the constituent sugar can be divided into two groups, inulins and levans. Inulins consist of 2,1-linked .beta.-fructofuranoside residues, whereas levans consist of 2,6-linked .beta.-fructofuranoside residues. Both can be linear or branched. The size of bacterial levans can vary from 20 kDa up to several MDa. There is limited information on the synthesis of levans. In most detail this synthesis has been studied in Zymomonas mobilis and in Bacillus species. Within **lactic acid bacteria, fructosyltransferases** have only been studied in streptococci. So far no **fructosyltransferases** have been reported in lactobacilli.

Summary of Invention Paragraph - BSTX (9):

[0008] Two novel genes encoding enzymes having **fructosyltransferase** activity have now been found in **Lactobacillus** reuteri, and their amino acid sequences have been determined. These are the first two enzymes identified in a **Lactobacillus** species capable of **producing a fructan**. One of the enzymes is an **inulosucrase** which produces a high molecular weight (>10.sup.7 Da) fructan containing .beta.(2-1) linked fructosyl units and fructo-oligosaccharides, while the other is a **levansucrase which produces a fructan** containing .beta.(2-6) linked fructosyl units. The invention thus pertains to the enzymes, to DNA encoding them, to recombinant cells containing such DNA and to their use in producing carbohydrates, as defined in the appending claims.

Summary of Invention Paragraph - BSTX (11):

[0009] It was found according to the invention that one of the novel

fructosyltransferases (FTFA; an **inulosucrase) produces a high molecular weight inulin** with .beta.(2-1) linked fructosyl units and fructo-oligosaccharides. The fructo-oligosaccharides synthesis was also observed in certain **Lactobacillus** strains, in particular in certain strains of **Lactobacillus** reuteri. However, the inulin has not been found in **Lactobacillus** reuteri culture supernatants, but only in extracts of E. coli cells expressing the above-mentioned **fructosyltransferase**. This **inulosucrase** consists of either 798 amino acids (2394 nucleotides) or 789 amino acids (2367 nucleotides) depending on the potential start codon used. The molecular weight (MW) deduced of the amino acid sequence of the latter form is 86 kDa and its isoelectric point is 4.51, at pH 7.

Summary of Invention Paragraph - BSTX (14):

[0012] **Fructosyltransferases** have been found in several bacteria such as Zymomonas mobilis, Erwinia amylovora, Acetobacter amylovora, Bacillus polymyxa, Bacillus amyloliquefaciens, Bacillus stearothermophilus, and Bacillus subtilis. In **lactic acid bacteria** this type of enzyme previously has only been found in some streptococci. Most bacterial **fructosyltransferases** have a molecular mass of 50-100 kDa (with the exception of the **fructosyltransferase** found in Streptococcus salivarius which has a molecular mass of 140 kDa). Amino acid sequence alignment revealed that the novel **inulosucrase** of lactobacilli has high homology with **fructosyltransferases** originating from Gram positive bacteria, in particular with Streptococcus enzymes. The highest homology (FIG. 2) was found with the SacB enzyme of Streptococcus mutans Ingbritt A (62% identity within 539 amino acids).

Summary of Invention Paragraph - BSTX (17):

[0015] A recombinant host cell, such as a mammalian (with the exception of human), plant, animal, fungal or bacterial cell, containing one or more copies of the nucleic acid construct mentioned above is an additional subject of the invention. The **inulosucrase** gene (starting at nucleotide 41) has been cloned in an E. coli expression vector under the control of an ara promoter in E. coli Top10. E. coli Top10 cells expressing the recombinant **inulosucrase** hydrolysed sucrose and **synthesized fructan** material. SDS-PAGE of arabinose induced E. coli Top10 cell extracts suggested that the recombinant **inulosucrase** has a molecular weight of 80-100 kDa, which is in the range of other known **fructosyltransferases** and in line with the molecular weight of 86 kDa deduced of the amino acid sequence depicted in FIG. 1.

Summary of Invention Paragraph - BSTX (18):

[0016] The invention further covers an **inulosucrase** according to the invention which, in the presence of sucrose, **produces a inulin** having .beta.(2-1)-linked D-fructosyl units and fructo-oligosaccharides. Two different types of fructans, inulins and levans, exist in nature. Surprisingly, the novel **inulosucrase** expressed in E. coli Top10 cell synthesizes a high molecular weight (>10.sup.7 Da) inulin and fructo-oligosaccharides, while in **Lactobacillus** reuteri culture supernatants, in addition to the fructo-oligosaccharides, a levan and not an inulin is found. This discrepancy can have several explanations: the **inulosucrase** gene may be

silent in **Lactobacillus** reuteri, or may not be expressed in **Lact bacillus** reuteri under the conditions tested, or the **inul sucrase** may only **synthesiz fruct - ligosaccharides in its natural h st, r the inulin** polymer may be degraded shortly after synthesis, or may not be secreted and remains cell-associated, or the **inulosucrase** may have different activities in **Lactobacillus** reuteri and E. coli Top10 cells.

Summary of Invention Paragraph - BSTX (19):

[0017] It was furthermore found according to the invention that certain lactobacilli, in particular **Lactobacillus** reuteri, possess another **fructosyltransferase, a levansucrase** (FTFB), in addition to the **inulosucrase** described above. The N-terminal amino acid sequence of the **fructosyltransferase** purified from **Lactobacillus** reuteri supernatant was found to be QVESNNYNGVAEVNTERQANGQI (SEQ ID No. 6). Furthermore, three internal sequences were identified, namely (M)(A)HLDVWDSWPVQDP(V) (SEQ ID No. 7), NAGSIFGT(K) (SEQ ID No. 8), V(E)(E)VYSPKVSTLMASDEVE (SEQ ID No. 9). The N-terminal amino acid sequence could not be identified in the deduced **inulosucrase** sequence. Also the amino acid sequences of the three internal peptide fragments of the purified **fructosyltransferase** were not present in the putative **inulosucrase** sequence. Evidently, the **inulosucrase** gene does not encode the purified **fructosyltransferase synthesizing the levan**. The complete amino acid sequence of the **levansucrase** is shown in SEQ ID No. 11 and the nucleotide sequence is shown in SEQ ID No. 10. The **levansucrase** comprises a putative membrane anchor (see amino acids 761-765 in SEQ ID No. 11) and a putative membrane spanning domain (see amino acids 766-787 in SEQ ID No. 11). The **fructan produced by the levansucrase** was identified in the **Lactobacillus** reuteri culture supematant as a linear (2.fwdarw.6)-.beta.-D-fructofuranan with a molecular weight of 150 kDa. The purified enzyme also **produces this fructan**.

Summary of Invention Paragraph - BSTX (20):

[0018] Additionally, the invention thus covers a protein having **levansucrase** activity with an amino acid identity of at least 65%, preferably at least 75%, and more preferably at least 85%, compared to the amino acid sequence of SEQ ID NO. 11. The second novel **fructosyltransferase produces a high molecular weight fructan** with .beta.(2-6) linked fructosyl units with sucrose or raffinose as substrate. The invention also covers a part of a protein with least 15 contiguous amino acids, which are identical to the corresponding part of the amino acid sequence of SEQ ID No. 11. A nucleotide sequence encoding any of the above-mentioned proteins, mutants, variants or parts thereof is a subject of the invention as well as a nucleic acid construct comprising the nucleotide sequence mentioned above operationally linked to an expression-regulating nucleic acid sequence. A recombinant host cell, such as a mammalian (with the exception of human), plant, animal, fungal or bacterial cell, containing one or more copies of the nucleic acid construct mentioned above is an additional subject of the invention. The invention further covers a protein according to the invention which, in the presence of sucrose, **produces a fructan** having .beta.(2-6)-linked D-fructosyl units.

Summary of Invention Paragraph - BSTX (21):

[0019] The invention also pertains to a process of **producing an inulin**-type and/or a levan-type of fructan as described above using **fructosyltransferases** according to the invention and a suitable fructose source such as sucrose, stachyose or raffinose. The fructans may either be produced by **Lactobacillus** strains or recombinant host cells according to the invention containing one or both **fructosyl transferases** or by a fuctosyltransferase enzyme isolated by conventional means from the culture of **fructosyltransferase**-positive lactobacilli, especially a **Lactobacillus** reuteri, or from a recombinant organism containing the **fructosyltransferase** gene or genes.

Summary of Invention Paragraph - BSTX (22):

[0020] Additionally, the invention concerns a process of producing fructo-oligosaccharides containing the characteristic structure of the fructans described above using a **Lactobacillus** strain or a recombinant host cell according to the invention containing one or both **fructosyltransferases** or an isolated **fructosyltransferase** according to the invention. There is a growing interest in oligosaccharides derived from homopolysaccharides, for instance for prebiotic purposes. Several fructo- and gluco-oligosaccharides are known to stimulate the growth of bifidobacteria in the human colon. Fructo-oligosaccharides produced by the **fructosyltransferase** described above are also part of the invention. Another way of producing fructo-oligosaccharides is by hydrolysis of the fructans described above. This hydrolysis can be performed by known hydrolysis methods such as enzymatic hydrolysis with enzymes such as levanase or inulinase or by acid hydrolysis. The fructo-oligosaccharides can also be produced in the presence of a **fructosyltransferase** according to the invention and an acceptor molecule such as lactose or maltose. The fructo-oligosaccharides to be produced according to the invention prefarably contain at least 2, more preferably at least 3, up to about 20 anhydrofructose units, optionally in addition to one or more other (glucose, galactose, etc.) units. These fructo-oligosaccharides are useful as prebiotics, and can be administered to a mammal in need of improving the bacterial status of the colon..

Summary of Invention Paragraph - BSTX (26):

[0024] Use of a **Lactobacillus** strain capable of **producing a levan,** inulin or fructo-oligosaccharides or a mixture thereof, as a probiotic, is also covered by the invention. Preferably, the **Lactobacillus** strain is also capable of producing a glucan, especially an 1,4/1,6-.alpha.-glucan as referred to above. The efficacy of some **Lactobacillus** reuteri strains as a probiotics has been demonstrated in various animals such as for instance poultry and humans. The administration of some **Lactobacillus** reuteri strains to pigs resulted in significantly lower serum total and LDL-cholesterol levels, while in children **Lactobacillus** reuteri is used as a therapeutic agent against acute diarrhea. For this and other reasons **Lactobacillus** reuteri strains, which were not reported to produce the glucans or fructans described herein, have been supplemented to commercially available probiotic products. The mode of action of **Lactobacillus** reuteri as a probiotic is still unclear. Preliminary studies indicated that gut colonization by **Lact bacillus** reuteri may be of importance. According to the invention, it was found that the mode of action of

Lact bacillus reuteri as a probiotic may reside partly in the ability to produce polysaccharides. **Lact bacillus** strains, preferably **Lactobacillus** reuteri strains, and more preferably **Lact bacillus** reuteri strain LB 121 and other strains containing one or more **fruct syltransferase** genes encoding proteins capable of producing inulins, levans and/or fructo-oligosaccharides can thus advantageously be used as a probiotic. They can also, together with these polysaccharides, be used as a symbiotic (instead of the term symbiotic, the term synbiotic can also be used). In that respect another part of the invention concerns a probiotic or symbiotic composition containing a **Lactobacillus** strain capable of **producing an inulin,** a levan or fructo-oligosaccharides and/or a glucan or a mixture thereof, said production being performed according to the process according to the invention. The probiotic or symbiotic compositions of the invention may be directly ingested with or without a suitable vehicle or used as an additive in conjunction with foods. They can be incorporated into a variety of foods and beverages including, but not limited to, yoghurts, ice creams, cheeses, baked products such as bread, biscuits and cakes, dairy and dairy substitute foods, confectionery products, edible oil compositions, spreads, breakfast cereals, juices and the like.

Detail Description Paragraph - DETX (3):

[0026] Isolation of DNA from **Lactobacillus** reuteri Nucleotide Sequence Analysis of the **Inulosucrase** (ftfA) Gene, Construction of Plasmids for Expression of the **Inulosucrase** Gene in E. coli Top10 Expression of the **Inulosucrase** gene in E. coli Top10 and Identification of the Produced Polysaccharides Produced by the Recombinant Enzyme.

Detail Description Paragraph - DETX (4):

[0027] General procedures for cloning, DNA manipulations and agarose gel electrophoresis were essentially as described by Sambrook et al. (1989) Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York. Restriction endonuclease digestions and ligations with T4 DNA ligase were performed as recommended by the suppliers. DNA was amplified by PCR techniques using ampliTAQ DNA polymerase (Perkin Elmer) or Pwo DNA polymerase. DNA fragments were isolated from agarose gels using the Qiagen extraction kit (Qiagen GMBH), following the instructions of the suppliers. **Lactobacillus** reuteri strain 121 (LMG 18388) was grown at 37.degree. C. in MRS medium (DIFCO) or in MRS-s medium (MRS medium containing 100 g/l sucrose instead of 20 g/l glucose). When fructo-oligosaccharides production was investigated phosphate was omitted and ammonium citrate was replaced by ammonium nitrate in the MRS-s medium. E. coli strains were grown aerobically at 37.degree. C. in LB medium, where appropriate supplemented with 50 .mu.g/ml ampicillin (for selection of recombinant plasmids) or with 0.02% (w/v) arabinose (for induction of the **inulosucrase** gene).

Detail Description Paragraph - DETX (6):

[0029] The **inulosucrase** gene was identified by amplification of chromosomal DNA of **Lactobacillus** reuteri with PCR using degenerated primers (5ftf, 6ftfi, and 12ftfi, see table 1) based on conserved amino acid sequences deduced from

different bacterial fructosyltranferase genes (SacB of Bacillus amyloliquefaciens, SacB of Bacillus subtilis, Streptococcus mutans **fruct syltransferase** and Streptococcus salivarius **fruct syltransferase**, see FIG. 4) and **Lactobacillus** reuteri DNA as template. Using primers 5ftf and 6fttfi, an amplification product with the predicted size of about 234 bp was obtained (FIG. 5A). This 234 bp fragment was cloned in E. coli JM109 using the pCR2.1 vector and sequenced. Transformations were performed by electroporation using the BioRad gene pulser apparatus at 2.5 kV, 25 .mu.F and 200 .OMEGA., following the instructions of the manufacturer. Sequencing was performed according to the method of Sanger et al. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467. Analysis of the obtained sequence data confirmed that part of a **fructosyltransferase** (ftf gene had been isolated. The 234 bp amplified fragment was used to design primers 7ftf and 8fttfi (see table 1). PCR with the primers 7ftf and 12fttfi gave a product of the predicted size of 948 bp (see FIG. 5B); its sequence showed clear similarity with previously characterized **fructosyltransferase** genes. The 948 bp amplified fragment was used to design the primers ftfAC1(i) and ftfAC2(i) (see table 1) for inverse PCR. Using inverse PCR techniques a 1438 bp fragment of the **inulosucrase** gene was generated, including the 3' end of the **inulosucrase** gene (see FIG. 5C). The remaining 5' fragment of the **inulosucrase** gene was isloated with a combination of standard and inverse PCR techniques. Briefly, **Lactobacillus** reuteri DNA was cut with restriction enzyme XhoI and ligated. PCR with the primers 7ftf and 8fttfi, using the ligation product as a template, yielded a 290 bp PCR product which was cloned into pCR2.1 and sequenced. This revealed that primer 8fttfi had annealed aspecifically as well as specifically yielding the 290 bp product (see FIG. 5D).

Detail Description Paragraph - DETX (7):

[0030] At this time, the N-terminal amino acid sequence of a **fructosyltransferase** enzyme (FTFB) purified from the **Lactobacillus** reuteri strain 121 was obtained. This sequence consisted of the following 23 amino acids: QVESNNYNGVAEVNTERQANGQI (SEQ ID No. 6). The degenerated primer 19fttf (YNGVAEV) was designed on the basis of a part of this N-terminal peptide sequence and primer 20fttfi was designed on the 290 bp PCR product. PCR with primers 19fttf and 20fttfi gave a 754 bp PCR product (see FIG. 5E), which was cloned into pCR2.1 and sequenced. Both DNA strands of the entire **fructosyltransferase** gene were double sequenced. In this way the sequence of a 2.6 kb region of the **Lactobacillus** reuteri DNA, containing the **inulosucrase** gene and its surroundings were obtained.

Detail Description Paragraph - DETX (8):

[0031] The plasmids for expression of the **inulosucrase** gene in E. coli Top10 were constructed as described hereafter. A 2414 bp fragment, containing the **inulosucrase** gene starting at the first putative start codon at position 41, was generated by PCR, using primers ftfA1 and ftfA2i. Both primers contained suitable restriction enzyme recognition sites (a NcoI site at the 5'end of ftfA1 and a BglII site at the 3'end of ftfA2i). PCR with **Lactobacillus** reuteri DNA, Pwo DNA polymerase and primers ftfA1 and ftfA2i yielded the complete **inulosucrase** gene flanked by NcoI and BglII restriction sites. The PCR product with blunt ends was ligated directly into pCRbluntII-Topo. Using the NcoI and

BglII restriction sites, the putative ftfA gene was cloned into the expression vector pBAD, downstream of the inducible arabinose promoter and in frame upstream of the Myc epitope and the His tag. The pBAD vector containing the **inul sucrase** gene (pSVH101) was transformed to E. coli Top10 and used to study **inulosucrase** expression. Correct construction of plasmid containing the complete **inulosucrase** gene was confirmed by restriction enzyme digestion analysis and by sequence analysis, showing an in frame cloning of the **inulosucrase** gene using the ribosomal binding site provided by the pBAD vector and the first putative start codon (at position 41) of **inulosucrase** (see FIG. 1).

Detail Description Paragraph - DETX (11):

[0034] **Fructan production by Lactobacillus** reuteri was studied with cells grown in MRS-s medium. Product formation was also studied with cell-free extracts of E. coli containing the novel **inulosucrase** incubated in reaction buffer (1 mg protein/10 ml buffer, incubated overnight at 37.degree. C.). Fructans were collected by precipitation with ethanol. .sup.1H-NMR spectroscopy and methylation analysis were performed as described by van Geel-Schutten et al. (1999) Appl. Environ. Microbiol. 65, 3008-3014. The molecular weights of the fructans were determined by high performance size exclusion chromatography coupled on-line with a multi angle laser light scattering and a differential refractive index detector.
Fructo-oligosaccharide synthesis was studied in **Lactobacillus** reuteri culture supernatants and in extracts of E. coli cells containing the novel **inulosucrase** incubated in reaction buffer (1 mg protein/10 ml buffer, incubated overnight at 37.degree. C.). Glucose and fructose were determined enzymatically as described above and fructo-oligosaccharides produced were analyzed using a Dionex column. The incubation mixtures were centrifuged for 30 min at 10,000.times.g and diluted 1:5 in a 100% DMSO solution prior to injection on a Dionex column. A digest of inulin (DP1-20) was used as a standard. Separation of compounds was achieved with anion-exchange chromatography on a CarboPac Pa1 column (Dionex) coupled to a CarboPac PA1 guard column (Dionex). Using a Dionex GP50 pump the following gradient was generated: % eluent B is 5% (0 min); 35% (10 min); 45% (20 min); 65% (50 min); 100% (54-60 min); 5% (61-65 min). Eluent A was 0.1 M NaOH and eluent B was 0.6 M NaAc in a 0.1 M NaOH solution. Compounds were detected using a Dionex ED40 electrochemical detector with an AU working electrode and a Ag/AgCl reference-electrode with a sensitivity of 300 nC. The pulse program used was: +0.1 Volt (0-0.4 s); +0.7 Volt (0.41-0.60 s); -0.1 Volt (0.61-1.00 s). Data were integrated using a Perkin Elmer Turbochrom data integration system. A different separation of compounds was done on a cation exchange column in the calcium form (Benson BCX4). As mobile phase Ca-EDTA in water (100 ppm) was used. The elution speed was 0.4 ml/min at a column temperature of 85.degree. C. Detection of compounds was done by a refractive index (Jasco 830-RI) at 40.degree. C. Quantification of compounds was achieved by using the software program Turbochrom (Perkin Elmer).

Detail Description Paragraph - DETX (20):

[0042] A **levansucrase** enzyme was purified from LB121 cultures grown on media containing maltose using ammonium sulfate precipitation and several

chromatography column steps (table 2). Maltose (glucose-glucose) was chosen because both **glucansucrase and levansucrase** can not use maltose as substrate. LB121 will grow on media containing maltose but will not produce polysaccharide. From earlier experiments it was clear that even with harsh methods the **levansucrase** enzyme could not be separated from its **pr duct levan**. These harsh methods included boiling the levan in a SDS solution and treating the levan with HCl and TFA. No levanase enzyme was commercially available for the enzymatic breakdown of levan. Only a single **levansucrase** was detected in maltose culture supernatants. In order to prove that the enzyme purified from maltose culture supernatant is the same enzyme which is responsible for the **levan production** during growth on raffinose, biochemical and biophysical tests were performed.

Detail Description Paragraph - DETX (40):

[0059] FIG. 1: SEQ ID No. 1; The deduced amino acid sequence of the novel **inulosucrase of Lactobacillus** reuteri (amino acid 1-789). Furthermore, the designations and orientation (<for 3' to 5' and >for 5' to 3') of the primers and the restriction enzymes used for (inverse) PCR, are shown at the right hand side. Putative start codons (ATG, at positions 41 and 68) and stop codon (TAA, at position 2435) are shown in bold. The positions of the primers used for PCR are shown in bold/underlined. The Nhel restriction sites (at positions 1154 and 2592) used for inverse PCR are underlined. The primers used and their exact posotions in the **inulosucrase** sequence are shown in table 1. Starting at amino acid 690, the 20 PXX repeats are underlined. At amino acid 755 the LPXTG motif is underlined.

Detail Description Paragraph - DETX (42):

[0061] FIG. 3: SEQ ID No. 2; The N-terminal and three internal amino acid sequences of the novel **levansucrase of Lactobacillus** reuteri.

Detail Description Paragraph - DETX (44):

[0063] FIG. 5: The strategy used for the isolation of the **inulosucrase** gene from **Lactobacillus** reuteri 121 chromosomal DNA.

Detail Description Table CWU - DETL (2):

2TABLE 2 Purification of the **Lactobacillus** reuteri LB 121 **levansucrase** (FTFB) enzyme Protein Total Specific Purification Yield Step (mg) Activity (U) Activity (U/mg) (fold) (%) Supernatant 128 64 0.5 1 100 Ammonium sulfate 35.2 42 1.2 2.4 65.6 precipitation (65%) Hydroxyl apatite 1.5 30.6 20.4 40.8 47.8 Phenyl superose 0.27 23 85 170 36 Gel Filtration 0.055 10 182 360 16 MonoQ 0.0255 4 176 352 6

Claims Text - CLTX (10):

9. A process of producing a **fructosyltransferase,** comprising culturing a host cell according to claim 8 or a **Lactobacillus** strain containing one or both **fructosyltransferases** according to claim 1 in a culture medium, and recovering the protein from the culture medium or the cell free extract.

US-PAT-NO: 6559356

DOCUMENT-IDENTIFIER: US 6559356 B1

TITLE: Nucleic acid molecules which encode proteins having **fructosyl transferase** activity and methods for **producing long-chain inulin**

DATE-ISSUED: May 6, 2003

INVENTOR-INFORMATION:

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APPL-NO: 09/ 565264

DATE FILED: May 5, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation of international application PCT/EP98/07115, filed Nov. 6, 1998, which designated the United States.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	197 49 122	November 6, 1997

US-CL-CURRENT: 800/284, 435/101 , 435/193 , 435/320.1 , 435/419 , 435/69.1 , 536/23.6 , 800/278 , 800/287 , 800/306 , 800/312 , 800/317.2 , 800/317.4 , 800/320 , 800/320.1 , 800/320.2 , 800/320.3

ABSTRACT:

Nucleic acid molecules which encode proteins having **fructosyl transferase** activity and methods for **producing long-chain inulin** Nucleic acid molecules are described encoding proteins with the enzymatic activity of a **fructosyl transferase**. These enzymes are **fructosyl transferases** (FFT). Moreover, vectors and host cells are described containing the nucleic acid molecules of the invention, in particular transformed plant cells, plant tissue and plants regenerable therefrom, which express the described FFT. Furthermore, methods for the **production of long-chain inulin** by using the described proteins, hosts, in particular the plant cells and/or FFT produced by them, are described.

29 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

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Abstract Text - ABTX (1):

Nucleic acid molecules which encode proteins having **fructosyl transferase** activity and methods for **producing long-chain inulin** Nucleic acid molecules are described encoding proteins with the enzymatic activity of a **fructosyl transferase**. These enzymes are **fructosyl transferases** (FFT). Moreover, vectors and host cells are described containing the nucleic acid molecules of the invention, in particular transformed plant cells, plant tissue and plants regenerable therefrom, which express the described FFT. Furthermore, methods for the **production of long-chain inulin** by using the described proteins, hosts, in particular the plant cells and/or FFT produced by them, are described.

TITLE - TI (1):

Nucleic acid molecules which encode proteins having **fructosyl transferase** activity and methods for **producing long-chain inulin**

Brief Summary Text - BSTX (2):

The present invention relates to nucleic acid molecules encoding proteins with the enzymatic activity of a **fructosyl transferase** (FFT). The invention also relates to vectors containing such nucleic acid molecules as well as to host cells transformed with said nucleic acid molecules, in particular plant cells, plant tissue and plants. Moreover, methods for the production of transgenic plants are described which **synthesize long-chain inulin** due to the introduction of nucleic acid molecules encoding an FFT. The present invention also relates to methods of **producing FFT and to the production of long-chain inulin** in various host organisms, in particular plants, as well as to in vitro methods for **producing long-chain inulin** by means of the FFT of the invention. The present invention further relates to the host cells of the invention and to the inulin obtainable by the processes of the present invention.

Brief Summary Text - BSTX (4):

So far only methods for **producing long-chain fructan** polysaccharides in plants have been described in which **fructosyl transferases** of bacterial origin are expressed. Most bacterial **fructosyl transferases synthesize levan,** a .beta.-2,6 linked fructosyl polymer which has numerous .beta.-2,1-branchings. Due to its numerous branchings levan has decisive disadvantages when it comes to technical processing and is therefore considerably less significant as a technical raw material then inulin. Up to now, only one bacterial gene is known, the gene **product of which is involved in the synthesis of inulin,** namely the ftf gene from Streptococcus mutans. It is in principle possible to express the gene in plants if the gene has previously been genetically engineered. However, the inulin yield obtained from transgenic plants is so low that the

economic utilization of the transgenic plants is out of question.

Brief Summary Text - BSTX (5):

Furthermore, a method for producing transgenic plants expressing **fruct syl transferases** from Helianthus tuberosus is known. The expression of these genes in transgenic plants leads to the **production of inulin** with an average degree of polymerization of DP=6 to DP=10. Polymers with this degree of polymerization may not be referred to as long-chain inulin. Inulin with an average DP=6 to DP=10 is unsuitable for most technical uses.

Brief Summary Text - BSTX (7):

PCT/US89/02729 describes the possibility of synthesizing carbohydrate polymers, in particular dextran or polyfructose, in transgenic plant cells, specifically in the fruits of transgenic plants. In order to **produce plants modified in such a way, the use of levan** sucrases from microorganisms, in particular from Aerobacter levanicum, Streptococcus salivarius and Bacillus subtilis, or of dextran sucrases from Leuconostoc mesenteroides is proposed. Neither the formation of the active enzymes nor that of **levan or dextran or the production** of transgenic plants is described. PCT/EP93/02110 discloses a method for producing transgenic plants expressing the lsc gene of the levan **sucrase** from the gram-negative bacterium Erwinia amylovora. The plants **produce a high-molecular, strongly branched levan**. PCT/NL93/00279 describes the transformation of plants with chimeric genes containing the sacB gene from Bacillus subtilis or the ftf gene from Streptococcus mutans. Transgenic plants expressing the sacB gene **produce a branched levan**. Plants expressing the ftf gene **synthesize high-molecular inulin**; the yield, however, is so low that an economic utilization is out of question. PCT/NL96/00012 discloses DNA sequences encoding enzymes synthesizing carbohydrate polymers as well as the production of transgenic plants by means of these DNA sequences. The disclosed sequences are derived from Helianthus tuberosus. According to PCT/NL96/00012, the disclosed sequences may be used in order to modify the fructan profile of petunia and potato, but also of Helianthus tuberosus itself. When expressing the SST and the FFT gene in transgenic plants, it is possible to **produce inulin**. The average degree of polymerization of inulin, however, ranges between DP=6 and DP=10. The **production of high-molecular inulin** is not possible by means of the method described in PCT/NL96/00012. PCT/EP97/02195 describes a method for **producing transgenic, inulin-producing** plants by means of the ftf gene from Streptococcus mutans. The yield of high-molecular inulin is low, as is the case with the plants described in PCT/NL9300279. DE 197 08 774.4 describes the **production of short-chain inulin** by means of enzymes exhibiting fructosyl polymerase activity. The short-chain **inulin may be produced** in transgenic plants. The yield of short-chain inuin is high and in potato it corresponds to the cellular content of sucrose. The **production of long-chain inulin,** however, is not described.

Brief Summary Text - BSTX (8):

The **synthesis of inulin** in plants has been thoroughly examined (Pollock & Chafterton, Fructans, The Biochemistry of Plants Vol. 14 (1988), Academic Press, pp.109-140). However, the inulin occurring naturally in plants is

short-chain fructan with a maximum degree of polymerization of approximately DP=35 (Pollock & Chatterton, 1988, loc.cit.). Synthesis and metabolism of fructans in plants are based on the activity of at least three enzymes: a sucrose-dependent sucrose-**fruct syl transferase** (SST) forming the tri-saccharide kestose, a fructan-dependent fructan-**fructosyl transferase** (FFT) which transfers fructosyl residues from fructan molecules with a minimum degree of polymerization of DP=3 (kestose) to sucrose and higher fructans, and a fructan exohydrolase (FEH) which removes fructose residues from fructan molecules. It is not known whether differences in the average molecular weight of the inulin in various plant species, for example about 2.times.10.sup.3 in the case of Allium cepa and 5.times.10.sup.3 in the case of Helianthus tuberosus, are based on the different properties of their SST, FFT or FEH.

Other Reference Publication - OREF (5):

Marcel Luscher et al., "**Inulin Synthesis** by a Combination of Purified **Fructosyltransferases** from Tubers of Helianthus tuberosus," FEBS Letters, 385, pp. 39-42 (1996).

US-PAT-NO: 6515203

DOCUMENT-IDENTIFIER: US 6515203 B1

TITLE: Nucleic acid molecules encoding enzymes having fructosyl polymerase activity

DATE-ISSUED: February 4, 2003

INVENTOR-INFORMATION:

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APPL-NO: 09/ 390224

DATE FILED: September 3, 1999

PARENT-CASE:

CROSS-REFERENCE TO A RELATED APPLICATION

This application is a continuation of copending international application PCT/EP98/01156, filed Mar. 2, 1998, which designated the United States and which is incorporated by reference herein.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	197 08 774	March 4, 1997

US-CL-CURRENT: 800/284, 435/101 , 435/193 , 435/320.1 , 435/419 , 435/468 , 435/69.1 , 536/23.6 , 800/278 , 800/287 , 800/317.2 , 800/320 , 800/320.1 , 800/320.2 , 800/320.3

ABSTRACT:

Described are nucleic acid molecules encoding enzymes having fructosyl polymerase activity. These enzymes are sucrose dependent sucrose fructosyltransferases (SST) enzymes. Furthermore, vectors and host cells are described containing the nucleic acid molecules, in particular transformed plant cells and plants that can be regenerated from them and that express the described SSTs. Furthermore, methods for the production of short-chain fructosyl polymers using the described hosts and/or the SSTs produced by them are described.

20 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

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Brief Summary Text - BSTX (4):

Up to now only processes for the production of long-chain fructane polysaccharides in plants by expression of enzymes of bacterial origin as well as a process for the production of transgenic plants expressing **fructosyltransferases** from Helianthus tuberosus have been described. Processes for the production of enzymes for producing short-chain fructosyl polymers are not known. In the specification of PCT/USA89/02729 the possibility to produce carbohydrate polymers, in particular dextrane or polyfructose, in transgenic plants, in particular in the fruits of transgenic plants, is described. For the **production of such modified plants the use of levan** sucrases from microorganisms, in particular from Aerobacter levanicum, Streptococcus salivarius and Bacillus subtilis, or from dextran sucrases from Leuconostoc mesenteroides are suggested. The **production of neither the active enzymes nor f levan** or dextrane nor of transgenic plants is described. The specification of PCT/EP93/02110 discloses a process for the production of transgenic plants expressing the Isc gene of levan **sucrase** from the gram-negative bacterium Erwinia amylovora. In the specification of PCT/NL93/00279 the transformation of plants having chimeric genes that contain the sacB gene from Bacillus subtilis or the ftf gene from Streptococcus mutans is described. In the case of the sacB gene a modification in the 5'-untranslated region of the gene is recommended in order to increase the expression level in transgenic plants. The specification of PCT/NL96/00012 discloses DNA sequences encoding the enzymes synthesizing carbohydrate polymers and the production of transgenic plants with the help of these DNA sequences. The disclosed sequences originate from Helianthus tuberosus. According to PCTL/NL96/00012 the disclosed sequences are not only suitable to modify the fructane profile of, for example, petunia and potato but also of Helianthus tuberosus itself. Therefore, the specification of PCT/NL96/100012 describes inter alia transgenic potato plants expressing an SST from Helianthus tuberosus. Even though the enzymatic activity of the SST expressed in the transgenic plants could be detected, only a low level of conversion of the substrate sucrose to short-chain fructosyl polymers could be achieved. This may be related to various factors, such as a low affinity of the enzyme to its substrate or a possible inhibition of the enzyme by the produced product.

Other Reference Publication - OREF (4):

Norbert Sprenger et al., "**Fructan Synthesis** in Transgenic Tobacco and Chicory Plants Expressing Bartley Sucrose: Fructan 6-**Fructosyltransferase,**" FEBS Letters, 400, pp. 355-358 (1997).

Other Reference Publication - OREF (5):

I. Vijn et al., "**Fructan of the Inulin Neoseries is Synthesized** in Transgenic Chicory Plants (Cichorium intybus L.) Harbouring Onion (Allium cepa L.) Fructan:Fructan 6G-**Fructosyltransferase,**" The Plant Journal, 11(3), pp.

387-398 (1997).

US-PAT-NO: 6501005

DOCUMENT-IDENTIFIER: US 6501005 B1

TITLE: DNA sequences which lead to the formation of polyfructans (levans), plasmids containing these sequences as well as a process for preparing transgenic plants

DATE-ISSUED: December 31, 2002

INVENTOR-INFORMATION:

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Willmitzer; Lothar	Berlin	N/A	N/A	DE

APPL-NO: 09/ 469648

DATE FILED: December 22, 1999

PARENT-CASE:

This application is a divisional of prior allowed application Ser. No. 08/943,374, filed Oct. 3, 1997 now U.S. Pat. No. 6,028,249, which in turn is a divisional application of U.S. Ser. No. 08/381,936, filed Feb. 9, 1995 now U.S. Pat. No. 5,792,923, as the National Phase of PCT/EP93/02110, filed Aug. 9, 1993, designating the U.S., published as WO 94/04692, claiming priority from German application P 42 27 061.8, filed Aug. 12, 1992; now U.S. Pat. No. 5,792,923.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	42 27 061	August 12, 1992

US-CL-CURRENT: 800/284, 435/419 , 435/430 , 435/468 , 800/288 , 800/298

ABSTRACT:

DNA which leads to the formation of polyfructans (levans), plasmids containing this DNA, as well as processes using plasmids for preparing transgenic plants with polyfructan (levan) expression.

10 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

---------- KWIC ----------

Brief Summary Text - BSTX (13):

Until now, genes for levan **sucrase** from Bacillus amyloliquefaciens (Tang et al. (1990) Gene 96, 89-93) and Bacillus subtilis (Steinmetz et al. (1985) Mol. Gen. Genetics 200, 220-228), have been described, and demonstrate relatively high homology with each other and both of which catalyze the **synthesis of fructans of the levan** type. Further, a **fructosyl transferase** from Streptococcus mutans (Shiroza et al. (1988) J. Bacteriology 170, 810-816) has been described. This shows little homology to either levan sucrases from Bacillus spp.. The fructan formed in Streptococcus mutans is of the inulin type.

Brief Summary Text - BSTX (28):

Since sucrose represents the substrate for the **levan sucrase, the production** of polyfructans is especially advantageous in those organs that store large amounts of sucrose. Such organs are for example, the roots of sugar beet or the stems of sugar cane. It is especially useful in genetically modified potatoes, which store sucrose in their tubers, through the blocking of starch biosynthesis.

US-PAT-NO: 6486314

DOCUMENT-IDENTIFIER: US 6486314 B1

TITLE: Glucan incorporating 4-, 6-, and 4, 6- linked anhydroglucose units

DATE-ISSUED: November 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Van Geel-Schutten; Gerritdina Hendrika	Driebergen-Rijsenburg	N/A	N/A	NL
Dijkhuizen; Lubbert	Zuidlaren	N/A	N/A	NL
Rahaoui; Hakim	Amersfoort	N/A	N/A	NL
Leer; Robert-Jan	Veenendaal	N/A	N/A	NL

APPL-NO: 09/ 604957

DATE FILED: June 28, 2000

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	00201871	May 25, 2000

US-CL-CURRENT: 536/123.12, 536/55.1

ABSTRACT:

The present invention describes a protein having glucosyltransferase activity. This protein is derived from lactobacilli, which are food-grade microorganisms with the Generally Recognized As Safe (GRAS) status. The protein produces a glucan with a unique structure having 4-linked, 6-lined and 4,6-linked anhydroglucose units or in the presence of suitable acceptors, oligosaccharides. According to the invention lactobacilli capable of producing this glucan using the novel glucosyltransferase can be used as a probiotic or symbiotic.

2 Claims, 15 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 15

---------- KWIC ---------

Brief Summary Text - BSTX (8):

It was found according to the invention that the glucans are produced by

certain **Lact bacillus** strains, in particular by certain strains of **Lactobacillus** reuteri, as a result of the activity of a single glucosyltransferase (**glucansucrase**).

Brief Summary Text - BSTX (29):

The glucosyltransferase (gtfA) gene was identified by amplification with PCR using degenerated primers (GTFpr1 (SEQ ID NO:14, 5' GAYAAKWSIAAKSYIRTIGTISARGC3' and GTFpr2 SEQ ID NO: 15, 5' GIKCICAIATRATRCCICTRIA3'; Y=T or C, K=G or T, W=A or T, S=C or G, R=A or G, I=A, C, G or T) based on conserved amino acid sequences deduced from different glucosyltransferase genes (gtfS of Streptococcus downei, gtfC of S. mutans, gtfI of S. downei, gtfK and gtfM of S. salivarius and dsrA of Leuconostoc mesenteroides) and **Lactobacillus** reuteri chromosomal DNA as template. An amplification project with the predicted size of about 660 bp was obtained (FIG. 1A). To investigate the possible presence of multiple copies of the glucosyltransferase gene, Southern hybridization was performed. DNA was restricted with endonucleases, separated by agarose gel electrophoresis and transferred to a Hybond nylon membrane. For hybridization probes were labelled with [.alpha.-.sup.32 P]dCTP using Random Primed DNA labeling kit (Boehringer Mannheim), following the manufacturer's instructions. The Southern hybridization of chromosomal DNA of the **Lactobacillus** reuteri strain 121 with the amplified 660 bp PCT fragment, followed by washing under non-stringent conditions (45.degree. C. 0.5 x SSC/0.1 SDS) revealed one hybridizing fragment, suggesting the presence of only a single copy of a glucosyltransferase gene in the **Lactobacillus** retueri strains. The 660 bp fragment was cloned in E. coli JM109 using the pCR2.1 vector. Transformations were performed by electroporation using the BioRad gene pulser apparatus at 2.5 kV, 25 .mu.F and 200 .OMEGA., following the instructions of the manufacturer. The fragment was sequenced by the method of Sanger et al. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467, confirming that the correct part of the gtfA gene had been isolated. The 660 bp amplified fragment was used to design primers for inverse PCR. Using inverse PCR techniques a 3 kb fragment of the isolated gtfA gene was generated (FIG. 1B). This 3 kb amplicon was identified by sequencing and probes were designed to isolate the EcoRI/Bg/II and EcoRI/HindIII fragments from a partial DNA library of **Lactobacillus** reuteri in E. coli DH5.alpha. (FIG. 1C). Positive clones were selected by colony blot hybridization using Hybond-N filters, following the instructions of the supplier and the cloned fragments were sequenced. Attempts to clone the C-terminal part of the **glucansucrase** gene in E. coli DH5.alpha. or JM109 using a partial DNA library strategy with different vectors failed. Therefore, the C-terminal part was isolated by inverse PCR. The remaining fragment, located between the EcoRI/Bg1II and EcoRI/HindIII fragments, was isolated by PCR techniques (FIG. 1D). The amplicons obtained were sequenced directly. To eliminate errors due to the PCR reaction, these fragments were sequenced for at least 4 times, using different clones per PCR reaction. Both DNA strands of the entire glucosyltransferase gene were sequenced twice. In this way the sequence of a 5.5 kb region of the **Lactobacillus** reuteri chromosomal DNA, containing the gtfA gene and its surroundings, were obtained.

Detailed Description Text - DETX (6):

FIG. 4: Alignment of catalytic cores of **alternansucrase** (ASR) of Leuconostoc mesenteroides strain NRRL B-1355 dextransuscrase (DSRS) of Leuconostoc mesenteroides strain NRRL B-512F, glucosyltransferase-D (GTFD) of Streptococcus mutans GS5, glucosyltransferase-A of **Lactobacillus** reuteri and **amyl sucrase** (AS) of Neisseria polysaccharea. indicates identical or conserved residues in all sequences); , gap in the sequence; AA amino acids which are conserved in all other glucosyltransferases but not in GTFA; , putative catalytic residues; , putative calcium binding sites; .diamond-solid., putative residues stabilizing the transition state; .gradient., residues possibly playing a role in binding of acceptor molecules and in the transfer of the glucosyl residue; .diamond., putative chloride binding sites; -Ex-, localization of .beta.-strands; -Hx-, localization of .alpha.-helices according to Mac Gregor et al. (1996) FEBS Let. 378, 262-266.

Other Reference Publication - OREF (1):
G.H. van Geel-Schutten et al., "Exopolysaccharide Production by **Lactobacillus** reuteri, Involving **Sucrase** Type of Enzymes, "Med. Fac. Landbouww, Univ. Gent, V. 65, No. 3a, 2000, pp. 197-201.

US-PAT-NO: 6468987

DOCUMENT-IDENTIFIER: US 6468987 B1

TITLE: Nutritional product for a person having ulcerative colitis

DATE-ISSUED: October 22, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Demichele; Stephen Joseph	Dublin	OH	N/A	N/A
Garleb; Keith Allen	Powell	OH	N/A	N/A
McEwen; John William	Gahanna	OH	N/A	N/A
Fuller; Martha Kay	Westerville	OH	N/A	N/A

APPL-NO: 09/ 395509

DATE FILED: September 14, 1999

PARENT-CASE:

This application is a divisional of application Ser. No. 09/083,736, filed on May 22, 1998, now U.S. Pat. No. 5,952,314, which is a continuation-in-part of application Ser. No. 08/221,349, filed on Apr. 1, 1994, now U.S. Pat. No. 5,780,451.

TABLE 1 CURRENT DRUG THERAPIES FOR ULCERATIVE COLITIS "non-specific therapies" DRUG ADMINISTRATION SIDE EFFECTS Anti-inflammatory agents Salicylates oral, rectal (enemas) secretory diarrhea, 5-ASA (ROWASA) nausea, headache, Sulfasalazine anemia, leakopenia Corticosteroids oral, topical, acne, weight gain, intravenous peptic ulcer, diabetes, glaucoma cataracts, osteoporosis, psychosis Immunosuppressive agents Azathioprine (AZA) oral, intravenous bone marrow 6-mercaptopurine suppression, Metronidazole infections, Cyclosporine pancreatitis Prednisone

US-CL-CURRENT: 514/54, 426/567 , 426/658 , 514/168 , 514/188 , 514/552 , 514/566 , 514/725 , 514/810 , 514/812 , 514/813 , 514/861

ABSTRACT:

An enteral nutritional product for a person having ulcerative colitis contains in combination (a) an oil blend which contains eicosapentaenoic acid (20:5n3) and/or docosahexaenoic acid (22:6n3), and (b) a source of indigestible carbohydrate which is metabolized to short chain fatty acids by microorganisms present in the human colon. Preferably the nutritional product also contains one or more nutrients which act as antioxidants.

41 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

---------- KWIC --------

Detailed Description Text - DETX (2):

In the first experiment the objective was to determine short chain fatty acid production from a variety of indigestible oligosaccharides during fermentation with mixed human fecal microbiota. Several indigestible oligosaccharides were tested including FOS, Raftilose.RTM. and XOS. FOS is a fructooligosaccharide produced on a commercial scale by fermenting granulated sucrose in water with a pure strain of Aspergillus niger. The organism produces a **fructosyltransferase** enzyme which links additional fructose units onto the fructose end of sucrose molecules to produce 1-kestose (GF.sub.2), nystose (GF.sub.3) and 1.sup.F -.beta.-fructo-furanosylnystose (GF.sub.4). Raftilose.RTM. is a fructooligosaccharide **produced via enzymatic hydrolysis of inulin,** which is marketed by Rhone-Poulenc (Raffinerie Tirlemontoise SA). The hydrolysis results in a wide array of oligosaccharides such as GF.sub.2, GF.sub.3 and GF.sub.4 as well as oligosaccharides containing just fructose (F.sub.3, F.sub.4, F.sub.5, etc.). XOS is a xylooligosaccharide produced via enzymatic hydrolysis of xylan. The primary ingredients of XOS are xylobiose, xylotriose and xylotetrose.

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  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 14:00:14 ON 19 JUN 2003

=> fil .bec,fsta
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
      ESBIOBASE, BIOTECHNO, WPIDS, FSTA' ENTERED AT 14:00:35 ON 19 JUN 2003
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

12 FILES IN THE FILE LIST

=> s fructosyltransferase# or fructosyl transferase# or inulinsucrase# or
levansucrase# or (inulin or levan)(w)sucrase#
FILE 'MEDLINE'
           134 FRUCTOSYLTRANSFERASE#
           191 FRUCTOSYL
         43869 TRANSFERASE#
            16 FRUCTOSYL TRANSFERASE#
                 (FRUCTOSYL(W)TRANSFERASE#)
             0 INULINSUCRASE#
           225 LEVANSUCRASE#
          7492 INULIN
           378 LEVAN
          2988 SUCRASE#
            15 (INULIN OR LEVAN)(W)SUCRASE#
L1          366 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
                # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'SCISEARCH'
           261 FRUCTOSYLTRANSFERASE#
           276 FRUCTOSYL
         36533 TRANSFERASE#
            89 FRUCTOSYL TRANSFERASE#
                 (FRUCTOSYL(W)TRANSFERASE#)
             1 INULINSUCRASE#
           288 LEVANSUCRASE#
          2812 INULIN
           400 LEVAN
          1772 SUCRASE#
             8 (INULIN OR LEVAN)(W)SUCRASE#
L2          580 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
                # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'LIFESCI'
           100 FRUCTOSYLTRANSFERASE#
            89 "FRUCTOSYL"
         12231 TRANSFERASE#
            24 FRUCTOSYL TRANSFERASE#
                 ("FRUCTOSYL"(W)TRANSFERASE#)
             0 INULINSUCRASE#
           161 LEVANSUCRASE#
           662 INULIN
           238 LEVAN
           362 SUCRASE#
             5 (INULIN OR LEVAN)(W)SUCRASE#
L3          264 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
                # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'BIOTECHDS'
           110 FRUCTOSYLTRANSFERASE#
```

```
            114 FRUCTOSYL
           2475 TRANSFERASE#
             49 FRUCTOSYL TRANSFERASE#
                  (FRUCTOSYL(W)TRANSFERASE#)
              0 INULINSUCRASE#
            177 LEVANSUCRASE#
            400 INULIN
            197 LEVAN
             80 SUCRASE#
              9 (INULIN OR LEVAN)(W)SUCRASE#
L4          312 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
                # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'BIOSIS'
            282 FRUCTOSYLTRANSFERASE#
            432 FRUCTOSYL
          68855 TRANSFERASE#
            146 FRUCTOSYL TRANSFERASE#
                  (FRUCTOSYL(W)TRANSFERASE#)
              0 INULINSUCRASE#
            269 LEVANSUCRASE#
           6573 INULIN
            711 LEVAN
           3346 SUCRASE#
             92 (INULIN OR LEVAN)(W)SUCRASE#
L5          692 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
                # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'EMBASE'
            116 FRUCTOSYLTRANSFERASE#
            107 "FRUCTOSYL"
          32982 TRANSFERASE#
              9 FRUCTOSYL TRANSFERASE#
                  ("FRUCTOSYL"(W)TRANSFERASE#)
              1 INULINSUCRASE#
            213 LEVANSUCRASE#
           7097 INULIN
            389 LEVAN
           1865 SUCRASE#
              6 (INULIN OR LEVAN)(W)SUCRASE#
L6          315 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
                # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'HCAPLUS'
            487 FRUCTOSYLTRANSFERASE#
            634 FRUCTOSYL
          43490 TRANSFERASE#
            117 FRUCTOSYL TRANSFERASE#
                  (FRUCTOSYL(W)TRANSFERASE#)
              2 INULINSUCRASE#
            493 LEVANSUCRASE#
           8657 INULIN
           1062 LEVAN
           3395 SUCRASE#
             69 (INULIN OR LEVAN)(W)SUCRASE#
L7          996 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
                # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'NTIS'
              2 FRUCTOSYLTRANSFERASE#
              2 FRUCTOSYL
           1080 TRANSFERASE#
              0 FRUCTOSYL TRANSFERASE#
                  (FRUCTOSYL(W)TRANSFERASE#)
```

```
             0 INULINSUCRASE#
             3 LEVANSUCRASE#
            71 INULIN
            16 LEVAN
            23 SUCRASE#
             0 (INULIN OR LEVAN)(W)SUCRASE#
L8           4 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
               # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'ESBIOBASE'
            96 FRUCTOSYLTRANSFERASE#
           112 FRUCTOSYL
         26814 TRANSFERASE#
            35 FRUCTOSYL TRANSFERASE#
                 (FRUCTOSYL(W)TRANSFERASE#)
             1 INULINSUCRASE#
           114 LEVANSUCRASE#
           903 INULIN
           127 LEVAN
           446 SUCRASE#
             3 (INULIN OR LEVAN)(W)SUCRASE#
L9         232 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
               # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'BIOTECHNO'
           115 FRUCTOSYLTRANSFERASE#
            98 FRUCTOSYL
         15499 TRANSFERASE#
            28 FRUCTOSYL TRANSFERASE#
                 (FRUCTOSYL(W)TRANSFERASE#)
             1 INULINSUCRASE#
           197 LEVANSUCRASE#
           841 INULIN
           215 LEVAN
           475 SUCRASE#
             3 (INULIN OR LEVAN)(W)SUCRASE#
L10        305 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
               # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'WPIDS'
            28 FRUCTOSYLTRANSFERASE#
           153 FRUCTOSYL
          4080 TRANSFERASE#
            71 FRUCTOSYL TRANSFERASE#
                 (FRUCTOSYL(W)TRANSFERASE#)
             0 INULINSUCRASE#
            19 LEVANSUCRASE#
           600 INULIN
           133 LEVAN
            98 SUCRASE#
            26 (INULIN OR LEVAN)(W)SUCRASE#
L11        124 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
               # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'FSTA'
            52 FRUCTOSYLTRANSFERASE#
           105 FRUCTOSYL
          1904 TRANSFERASE#
            31 FRUCTOSYL TRANSFERASE#
                 (FRUCTOSYL(W)TRANSFERASE#)
             0 INULINSUCRASE#
            91 LEVANSUCRASE#
           679 INULIN
           139 LEVAN
```

```
          88 SUCRASE#
           4 (INULIN OR LEVAN)(W)SUCRASE#
L12      165 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
             # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

TOTAL FOR ALL FILES
L13     4355 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
             # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W) SUCRASE#

=> s l13 and (lactobacillus or lactic acid bacteri?)
FILE 'MEDLINE'
         10523 LACTOBACILLUS
         27619 LACTIC
       1183408 ACID
        532117 BACTERI?
          1730 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L14        5 L1 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'SCISEARCH'
          9409 LACTOBACILLUS
         20032 LACTIC
        919196 ACID
        284895 BACTERI?
          5480 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L15        8 L2 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'LIFESCI'
          5351 LACTOBACILLUS
          6298 "LACTIC"
        260919 "ACID"
        161467 BACTERI?
          2424 LACTIC ACID BACTERI?
                 ("LACTIC"(W)"ACID"(W)BACTERI?)
L16        6 L3 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'BIOTECHDS'
          2373 LACTOBACILLUS
          4937 LACTIC
        100678 ACID
        101276 BACTERI?
          2565 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L17       10 L4 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'BIOSIS'
         15182 LACTOBACILLUS
         26717 LACTIC
       1122724 ACID
        779123 BACTERI?
          4925 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L18        8 L5 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'EMBASE'
          7862 LACTOBACILLUS
         32128 "LACTIC"
       1147108 "ACID"
        388754 BACTERI?
          1821 LACTIC ACID BACTERI?
                 ("LACTIC"(W)"ACID"(W)BACTERI?)
L19        9 L6 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)
```

```
FILE 'HCAPLUS'
         18535 LACTOBACILLUS
         80839 LACTIC
       3656729 ACID
        498993 BACTERI?
          7978 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L20          12 L7 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'NTIS'
           110 LACTOBACILLUS
           560 LACTIC
         42987 ACID
         18022 BACTERI?
            33 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L21           0 L8 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'ESBIOBASE'
          3247 LACTOBACILLUS
          4578 LACTIC
        254386 ACID
        140560 BACTERI?
          1732 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L22           4 L9 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'BIOTECHNO'
          4762 LACTOBACILLUS
          7837 LACTIC
        328601 ACID
        179059 BACTERI?
          1982 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L23           7 L10 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'WPIDS'
          3077 LACTOBACILLUS
         12976 LACTIC
        789386 ACID
         86109 BACTERI?
          1950 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L24           6 L11 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'FSTA'
          8805 LACTOBACILLUS
         14350 LACTIC
        108568 ACID
         59097 BACTERI?
          6253 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L25           4 L12 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

TOTAL FOR ALL FILES
L26          79 L13 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

=> s (fructan or levan or inulin)(5a)(mak###### or produc? or synthes?)
FILE 'MEDLINE'
           165 FRUCTAN
           378 LEVAN
          7492 INULIN
        244189 MAK######
       1087241 PRODUC?
```

```
        427516 SYNTHES?
L27        216 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
               ?)

FILE 'SCISEARCH'
           581 FRUCTAN
           400 LEVAN
          2812 INULIN
        266271 MAK######
       1458879 PRODUC?
        739600 SYNTHES?
L28        429 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
               ?)

FILE 'LIFESCI'
           120 FRUCTAN
           238 LEVAN
           662 INULIN
         44771 MAK######
        447263 PRODUC?
        126867 SYNTHES?
L29        222 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
               ?)

FILE 'BIOTECHDS'
            83 FRUCTAN
           197 LEVAN
           400 INULIN
          8610 MAK######
        181090 PRODUC?
         25939 SYNTHES?
L30        278 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
               ?)

FILE 'BIOSIS'
           773 FRUCTAN
           711 LEVAN
          6573 INULIN
        162874 MAK######
       1486678 PRODUC?
        585019 SYNTHES?
L31        646 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
               ?)

FILE 'EMBASE'
           326 FRUCTAN
           389 LEVAN
          7097 INULIN
        211872 MAK######
       1050887 PRODUC?
        523551 SYNTHES?
L32        252 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
               ?)

FILE 'HCAPLUS'
          1048 FRUCTAN
          1062 LEVAN
          8657 INULIN
        495108 MAK######
       3674763 PRODUC?
        759216 PRODN
       4047742 PRODUC?
                 (PRODUC? OR PRODN)
       1297112 SYNTHES?
```

```
L33        1176 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
                ?)

FILE 'NTIS'
              3 FRUCTAN
             16 LEVAN
             71 INULIN
         114868 MAK######
         356340 PRODUC?
          41089 SYNTHES?
L34           5 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
                ?)

FILE 'ESBIOBASE'
            255 FRUCTAN
            127 LEVAN
            903 INULIN
          50519 MAK######
         425388 PRODUC?
         148053 SYNTHES?
L35         192 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
                ?)

FILE 'BIOTECHNO'
            215 FRUCTAN
            215 LEVAN
            841 INULIN
          32123 MAK######
         373674 PRODUC?
         162184 SYNTHES?
L36         200 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
                ?)

FILE 'WPIDS'
            126 FRUCTAN
            133 LEVAN
            600 INULIN
         558735 MAK######
        1945594 PRODUC?
         106127 SYNTHES?
L37         157 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
                ?)

FILE 'FSTA'
            137 FRUCTAN
            139 LEVAN
            679 INULIN
          16546 MAK######
         265020 PRODUC?
          10572 SYNTHES?
L38         291 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
                ?)

TOTAL FOR ALL FILES
L39        4064 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
                ?)

=> s l13(5a)l39
FILE 'MEDLINE'
L40          26 L1 (5A)L27

FILE 'SCISEARCH'
L41          48 L2 (5A)L28
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FILE 'LIFESCI'
L42          20 L3 (5A)L29

FILE 'BIOTECHDS'
L43          27 L4 (5A)L30

FILE 'BIOSIS'
L44          71 L5 (5A)L31

FILE 'EMBASE'
L45          22 L6 (5A)L32

FILE 'HCAPLUS'
L46         103 L7 (5A)L33

FILE 'NTIS'
L47           0 L8 (5A)L34

FILE 'ESBIOBASE'
L48          30 L9 (5A)L35

FILE 'BIOTECHNO'
L49          28 L10(5A)L36

FILE 'WPIDS'
L50          14 L11(5A)L37

FILE 'FSTA'
L51          26 L12(5A)L38

TOTAL FOR ALL FILES
L52         415 L13(5A) L39

=> s (l26 or l52) not 2002-2003/py
FILE 'MEDLINE'
        754471 2002-2003/PY
L53          25 (L14 OR L40) NOT 2002-2003/PY

FILE 'SCISEARCH'
       1325748 2002-2003/PY
L54          43 (L15 OR L41) NOT 2002-2003/PY

FILE 'LIFESCI'
        110034 2002-2003/PY
L55          22 (L16 OR L42) NOT 2002-2003/PY

FILE 'BIOTECHDS'
         28410 2002-2003/PY
L56          31 (L17 OR L43) NOT 2002-2003/PY

FILE 'BIOSIS'
        672395 2002-2003/PY
L57          70 (L18 OR L44) NOT 2002-2003/PY

FILE 'EMBASE'
        601108 2002-2003/PY
L58          24 (L19 OR L45) NOT 2002-2003/PY

FILE 'HCAPLUS'
       1480932 2002-2003/PY
L59          88 (L20 OR L46) NOT 2002-2003/PY

FILE 'NTIS'
         13938 2002-2003/PY
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L60           0 (L21 OR L47) NOT 2002-2003/PY

FILE 'ESBIOBASE'
        388954 2002-2003/PY
L61          26 (L22 OR L48) NOT 2002-2003/PY

FILE 'BIOTECHNO'
        153285 2002-2003/PY
L62          28 (L23 OR L49) NOT 2002-2003/PY

FILE 'WPIDS'
       1448234 2002-2003/PY
L63          14 (L24 OR L50) NOT 2002-2003/PY

FILE 'FSTA'
         27984 2002-2003/PY
L64          21 (L25 OR L51) NOT 2002-2003/PY

TOTAL FOR ALL FILES
L65         392 (L26 OR L52) NOT 2002-2003/PY

=> fil .becpat
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                 ENTRY    SESSION
FULL ESTIMATED COST                              27.94      28.15

FILES 'BIOTECHDS, HCAPLUS, WPIDS' ENTERED AT 14:11:16 ON 19 JUN 2003
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

3 FILES IN THE FILE LIST

=> s (126 or 152) and wo/pc and 2002-2003/py
FILE 'BIOTECHDS'
          46092 WO/PC
          28410 2002-2003/PY
L66           0 (L17 OR L43) AND WO/PC AND 2002-2003/PY

FILE 'HCAPLUS'
         313791 WO/PC
        1480932 2002-2003/PY
L67           9 (L20 OR L46) AND WO/PC AND 2002-2003/PY

FILE 'WPIDS'
         749705 WO/PC
        1448234 2002-2003/PY
L68           3 (L24 OR L50) AND WO/PC AND 2002-2003/PY

TOTAL FOR ALL FILES
L69          12 (L26 OR L52) AND WO/PC AND 2002-2003/PY

=> dup rem
ENTER L# LIST OR (END):169
PROCESSING COMPLETED FOR L69
L70           9 DUP REM L69 (3 DUPLICATES REMOVED)

=> d tot

L70  ANSWER 1 OF 9  HCAPLUS  COPYRIGHT 2003 ACS
TI   Fructan production by transgenic beets expressing two different
     fructosyltransferases
SO   PCT Int. Appl., 26 pp.
     CODEN: PIXXD2
IN   Weynes, Guy; Lathouwers, Jean; Van Dun, Kees
AN   2003:6097  HCAPLUS
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DN 138:50882

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003000854	A2	20030103	WO 2002-US19860	20020625 <--

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

L70 ANSWER 2 OF 9 HCAPLUS COPYRIGHT 2003 ACS

TI Counter selection strategy for gram-negative bacteria used in plant transformation

SO PCT Int. Appl., 18 pp.
CODEN: PIXXD2

IN Farrand, Stephen K.; Staswick, Paul E.; Clemente, Thomas E.

AN 2002:123230 HCAPLUS

DN 136:178942

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002012519	A2	20020214	WO 2001-US41618	20010807 <--
WO 2002012519	A3	20030206		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

AU 2001083547	A5	20020218	AU 2001-83547	20010807 <--
US 2002061579	A1	20020523	US 2001-924101	20010807 <--

L70 ANSWER 3 OF 9 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 1

TI Purification, characterization and use of inulosucrase and **levansucrase** from **Lactobacillus** reuteri

SO PCT Int. Appl., 54 pp.
CODEN: PIXXD2

IN Van Geel-Schutten, Gerritdina Hendrika; Rahaoui, Hakim; Dijkhuizen, Lubbert; Van Hijum, Sacha Adrianus Fokke Taco

AN 2001:868644 HCAPLUS

DN 136:17259

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001090319	A2	20011129	WO 2001-NL392	20010523 <--

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1283888	A2	20030219	EP 2001-934630	20010523 <--

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR

L70 ANSWER 4 OF 9 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 2
TI Enzymatic production of difructose dianhydride IV from sucrose and relevant enzymes and genes coding for them
SO PCT Int. Appl., 72 pp.
CODEN: PIXXD2
IN Rhee, Sangki; Song, Kibang; Kim, Chulho; Ryu, Eunja; Lee, Yongbok
AN 2001:300842 HCAPLUS
DN 134:339638

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
PI WO 2001029185	A1	20010426	WO 2000-KR1183	20001019	<--
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM					
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG					
EP 1141236	A1	20011010	EP 2000-976414	20001019	
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO					
JP 2003512045	T2	20030402	JP 2001-532170	20001019	<--

L70 ANSWER 5 OF 9 HCAPLUS COPYRIGHT 2003 ACS
TI Nucleotide coding for Aspergillus sydowi fructosyltransferase, isolation, expression and application for the production of inulin-type polyfructoses
SO Ger. Offen., 28 pp.
CODEN: GWXXBX
IN Heyer, Arnd; Rehm, Jochen; Wendenburg, Regina
AN 2000:157875 HCAPLUS
DN 132:204850

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
PI DE 19840028	A1	20000309	DE 1998-19840028	19980902	
CA 2342115	AA	20000316	CA 1999-2342115	19990827	
WO 2000014246	A1	20000316	WO 1999-EP6319	19990827	<--
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM					
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG					
AU 9959714	A1	20000327	AU 1999-59714	19990827	
BR 9913390	A	20010522	BR 1999-13390	19990827	
EP 1108037	A1	20010620	EP 1999-968682	19990827	
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI					
JP 2002524079	T2	20020806	JP 2000-568987	19990827	<--
US 2002064850	A1	20020530	US 2001-798791	20010302	<--

L70 ANSWER 6 OF 9 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 3
TI A novel fructosyl transferase isolated from artichoke, and its use in the production of long-chain inulin
SO PCT Int. Appl., 50 pp.
CODEN: PIXXD2
IN Heyer, Arnd G.; Hellwege, Elke W.; Gritscher, Dominique
AN 1999:326065 HCAPLUS
DN 130:348208

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

PI	WO 9924593	A1	19990520	WO 1998-EP7115	19981106 <--
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	DE 19749122	A1	19990610	DE 1997-19749122	19971106
	ZA 9810110	A	19990505	ZA 1998-10110	19981105
	CA 2309133	AA	19990520	CA 1998-2309133	19981106
	AU 9916674	A1	19990531	AU 1999-16674	19981106 <--
	AU 753390	B2	20021017		
	EP 1029067	A1	20000823	EP 1998-961152	19981106
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	BR 9813968	A	20000926	BR 1998-13968	19981106
	JP 2001521757	T2	20011113	JP 2000-519587	19981106
	US 6559356	B1	20030506	US 2000-565264	20000505 <--

L70 ANSWER 7 OF 9 HCAPLUS COPYRIGHT 2003 ACS
TI Functional sugar polymers from inexpensive sugar sources and apparatus for preparing same
SO PCT Int. Appl., 41 pp.
CODEN: PIXXD2
IN Catani, Steven J.; Laurenzo, Kathleen S.; Navia, Juan L.; Walkup, Robert E.
AN 1999:723198 HCAPLUS
DN 131:321633

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9957300	A1	19991111	WO 1999-US9752	19990504 <--
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	AU 9937858	A1	19991123	AU 1999-37858	19990504 <--
	AU 759465	B2	20030417		
	BR 9911012	A	20010206	BR 1999-11012	19990504
	EP 1076716	A1	20010221	EP 1999-920336	19990504
	R: DE, DK, FR, GB, IT, SE				
	US 6423833	B1	20020723	US 1999-305788	19990504 <--
	NO 2000005545	A	20001103	NO 2000-5545	20001103
	US 2003044942	A1	20030306	US 2002-127462	20020423 <--

L70 ANSWER 8 OF 9 HCAPLUS COPYRIGHT 2003 ACS
TI Novel cellulose-producing bacteria
SO PCT Int. Appl., 51 pp.
CODEN: PIXXD2
IN Tsuchida, Takayasu; Tonouchi, Naoto; Seto, Akira; Kojima, Yukiko; Matsuoka, Masanobu; Yoshinaga, Fumihiro
AN 1997:717991 HCAPLUS
DN 127:328870

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9740135	A1	19971030	WO 1997-JP514	19970224 <--
	W: JP, KR, US				
	RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				

EP 834555	A1	19980408	EP 1997-904620	19970224	
R: DE, FR, GB					
JP 3341017	B2	20021105	JP 1997-523517	19970224	<--
US 5962278	A	19991005	US 1998-973757	19980203	
US 6110712	A	20000829	US 1999-274470	19990323	

L70 ANSWER 9 OF 9 HCAPLUS COPYRIGHT 2003 ACS
TI Preparation of transgenic plants with modified fructan patterns
SO PCT Int. Appl., 43 pp.
CODEN: PIXXD2
IN Smeekens, Josephus Christianus Maria; Ebskamp, Michael Johannes Marcus; Weisbeek, Petrus Jacobus
AN 1994:626648 HCAPLUS
DN 121:226648

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
PI WO 9414970	A1	19940707	WO 1993-NL279	19931228	<--
W: AU, BB, BG, BR, BY, CA, CZ, FI, HU, JP, KP, KR, KZ, LK, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SE, SK, UA, US, VN					
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG					
NL 9300646	A	19940718	NL 1993-646	19930415	
AU 9458437	A1	19940719	AU 1994-58437	19931228	
EP 677112	A1	19951018	EP 1994-904344	19931228	<--
EP 677112	B1	20030326			
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE					
HU 71782	A2	19960228	HU 1995-1912	19931228	
JP 08507918	T2	19960827	JP 1993-515042	19931228	
PL 178789	B1	20000630	PL 1993-309606	19931228	
RU 2152997	C2	20000720	RU 1995-115683	19931228	
AT 235557	E	20030415	AT 1994-904344	19931228	<--
US 6025542	A	20000215	US 1995-446834	19950803	
US 5986173	A	19991116	US 1998-59484	19980413	

=> log y
COST IN U.S. DOLLARS

	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	36.90	65.05

STN INTERNATIONAL LOGOFF AT 14:17:13 ON 19 JUN 2003